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Studies on *Oscinella frit* Linn.

Notes on the resistance of cereals to infestation

BY NORMAN CUNLIFFE, M.A., D.Sc. AND D. J. HODGES, B.Agr. (Queen's University)
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(With 1 Text-figure)

Resistance of the oat plant to infestation by the frit fly is not correlated with either carbohydrate or silica content. By increasing the number of eggs placed on an oat variety, proved to be resistant in the field, its resistance can be reduced below that of the most susceptible type, the inference being that resistance is indirect and due to some form of plant attractiveness which determines extent of oviposition. Oviposition studies in the field show that oviposition on susceptible varieties of oats, such as Victory and Star, is three times as great as on resistant types, such as Summer and Spet; selections from crosses between susceptible and resistant types, intermediate in resistance, receive a proportionate number of eggs, resistance being inherited. Some oviposition data for wheat and barley are included. Fly orientation is not controlled by either chemotaxis or hydrotaxis, but is influenced by stereotaxis; negative results have so far been obtained from phototaxis studies. The resistance to infestation in the field of twenty-two named varieties of oats is compared with that of a standard variety; none is so resistant as Summer, while Richland Iowa 411 and Eagle approach Sandford in possessing moderate resistance. The marked difference between Victory and Summer in regard to stem infestation in spring is not extended to the grain infestation, no marked difference being observed. Susceptibility to sterility of grain shows significant differences, and there is an indication that segregation may be transgressive. Late nitrogenous dressings have no effect on extent of either grain infestation or sterility. The recovery capacity of Sandford almost equals that of Gris de Houdan, a good tillering variety. Maize exhibits varietal differences in extent of resistance to attack. The protective effect of D.D.T. is considerable, but not complete.

CARBOHYDRATE AND SILICA CONTENT OF THE OAT PLANT IN RELATION TO RESISTANCE TO ATTACK

In a previous paper (Cunliffe, 1936) it was suggested that differences in extent of attack on oat varieties might be due to varietal differences in carbohydrate production or deposition of silica, each tending to increase the difficulty of larval travel and, therefore, the resistance of the plant. In that investigation a small but significant difference in percentage crude-fibre content and a marked significant difference in total carbohydrate produced per unit content of nitrogen were observed on comparing Victory oat and a Spet × Star oat cross. Differences in silica content were suggestive, but not significant.

In view of the fact that resistance always seemed to be associated closely with growth, it was considered during 1936 that a repetition of these experiments on a wider statistical basis was justified.*

Percentage of crude fibre and carbohydrate/nitrogen ratio

A Latin square experiment was laid down on 20 February for sampling at various stages of plant growth, using related varieties of oats, namely, one strain each of the crosses Spet × Star (Sandford) and Summer × Star, the resistant parent varieties Summer and Spet and the susceptible varieties Star and Victory. The plots were sampled when the plants were in the late 2-leaf stage (without tillers), in the early 5-leaf stage (average tiller dry matter, 2·76 g./100 plants) and again in the 7-leaf stage (average tiller dry matter, 23·5 g./100 plants). The 2-leaf stage samples only provided sufficient material for the silica analysis. The results of primary shoot analyses are given in Table 1.

The selections yielded more crude fibre than the respective parent types although only intermediate in resistance, while the resistant parents differed in their relation to the susceptible variety, Star. Thus, variation in percentage of crude fibre does not offer any explanation of varietal differences in resistance.

The data set out in Table 1 also show that a high figure for the carbohydrate/nitrogen ratio is not correlated with resistance, both Spet and Summer being more resistant to attack than the selections Spet × Star and Summer × Star, and both these selections being, in turn, more resistant than Victory and Star.

Percentage of combined silica in primary shoots at various leaf stages

Samples for the estimation of combined silica were taken from the same Latin square, and the results of

* The essential statistical analyses have been lodged with the Librarian of the British Museum. A statement of significance of result means significance at the 1% point of distribution.

primary shoot analysis are given in Table 1. Summer is outstanding, being significantly above Spet, the other resistant type; each contains more combined silica in the later stages than either Victory or Star. But, whereas the selection Spet × Star approaches its resistant parent, the equally resistant selection Summer × Star tends towards its susceptible parent. The inference is that content of combined silica is not correlated with resistance.

This is confirmed by soil treatment; sodium silicate was used to provide additional silica while manganese sulphate, possibly a catalytic fertilizer, was used to complete the square; dressings of sodium silicate at the rate of 4 cwt./acre, or manganese sulphate at the rate of ½ cwt./acre, were made a week after sowing Victory and Summer in Latin square formation; sowing was deferred until 21 April to ensure a heavy attack. During growth, it was not possible to distinguish between treated and control plots. The data given in Table 2 show that the silicate dressing significantly reduced the extent of attack on Summer by 9%, but that it was completely ineffective in the case of Victory, although this might be explained by the high incidence of attack. But chemical analysis of whole-plant samples (Table 2) failed to show any significant differences in percentage of combined silica. Repetitions of this experiment in subsequent years, using other varieties as well as Summer and Victory, gave similar results. Spikelet production per plant of the stand on 20 May was necessarily low owing to the abnormal sowing date resulting in an abnormally heavy attack, but silica failed to have any influence whatsoever on spikelet production and presumably therefore on tiller infestation, under conditions when it might have been expected to be effective.

It seems evident, therefore, that no correlation between percentage of crude fibre, carbohydrate/nitrogen ratio or percentage of combined silica and resistance to attack exists.

Indirect Resistance of the Oat Plant to Infestation

No evidence of direct resistance to attack having been found, the question arises, is resistance in fact indirect? If so, it should be possible, by increasing the number of eggs placed on the resistant type, to make it apparently more susceptible than the susceptible type. When making the experiment it is necessary to ensure (*a*) that oviposition is natural, (*b*) that control plots can be provided to measure natural infestation and (*c*) to know when to sow to obtain natural infestation in the middle of the possible range of attack and yet to avoid extreme natural infestation.

First, then, the experiment must be made under natural field conditions. Secondly, the most intimate mixture of plant to enforce the greatest display of natural choice of oviposition site by the fly is provided by alternate seeding in the rows within the plot, so that each plant of the one variety is surrounded by plants of the other. Under these conditions a significant varietal difference in extent of infestation is maintained; for example, during 1936, Summer and Victory, sown on 26 April, 2 × 6 in. in five plots each of 150 seeds, showed the following mean percentage losses of primary shoots, namely, $61{\cdot}8 \pm 7{\cdot}56$ and $96{\cdot}5 \pm 0{\cdot}63$ respectively; a repetition, using ten plots each of 300 seeds, sown on 21 April 1937, gave means of 59·5 and 88·7%, the difference of 29·2 being clearly significant. The correlation coefficient $r = +0{\cdot}559 \pm 0{\cdot}146$ for the percentages of primary shoot infestation, for Victory and Summer, shows the extent of the irregularity of infestation. Incidentally, no significant correlation coefficients were obtained from the 1937 data for (*a*) dry matter in unattacked primary shoots, Victory and Summer ($r = +0{\cdot}518 \pm 0{\cdot}155$), and (*b*) percentage infestation and dry matter in unattacked primary shoots of Summer ($r = +0{\cdot}220 \pm 0{\cdot}201$). Finally, a Latin square plot laid down during 1936 to show that inherited resistance under the conditions of moderate attack would have a commercial value greater than might be indicated by data obtained when the attack was intense also related sowing date and extent of infestation (see Table 3), providing data on which the following experiments were based.

At a date normally suitable to ensure considerable but not extreme natural infestation, namely, about the end of March (see Table 3), such sowings of resistant and susceptible types have been made in the field for several seasons, and the effect of an increase in egg deposition on the resistant type estimated. Rain, wind, infertility, scarcity of eggs on convenient collecting surfaces, plant losses, etc., each tends towards depression of effect. Eggs attached to the coleoptile are normally difficult to find at the beginning of May (5 per 200 plants is not abnormally low), while when eggs are plentiful at the end of the month plants in the 1- and 2-leaf stages are liable to show 100% natural infestation.

(1) 1939. Sowings of Summer and Victory for treatment and control were made on 31 March; additional egg deposition was made from eggs attached to the coleoptile to avoid egg injuries, the coleoptile segment being placed between the coleoptile and primary shoot of the plant to be treated. Later, additional eggs were placed on apparently unattacked plants and infestation after 3 weeks was as follows:

	Treated plot		Control plot	
	No. of plants	Loss of primary shoots %	No. of plants	Loss of primary shoots %
Victory	41	29	57	9
Summer	37	24	65	0

TABLE 1. *Crude fibre, carbohydrate/nitrogen and silica*

	Stage	Summer × Star	Summer	Spet × Star	Spet	Victory	Star	Sig. diff.
Crude fibre (%)	7-leaf	20·25	19·76	19·69	18·89	18·47	18·27	0·68
	5-leaf	16·04	15·96	15·46	15·19	14·75	15·42	0·65
Total carbohydrate per unit content of nitrogen	7-leaf	14·28	13·61	15·30	13·20	13·37	13·48	0·99
	5-leaf	11·28	11·50	11·19	10·90	10·58	10·76	0·44
Combined silica (%)	7-leaf	9·23	10·93	9·96	10·34	8·92	8·75	0·53
	5-leaf	9·68	11·27	9·63	9·70	9·11	9·14	0·61
	2-leaf	8·38	9·05	8·01	8·17	8·35	8·01	Not sig.

TABLE 2. *Silica treatment*

	Control	$MnSO_4$	Na_2SiO_3
	Individual treatments: loss of primary shoots by frit attack (%)		
Summer	72·6	67·7	63·6
Victory	98·8	97·8	98·8
	Sig. diff. 5·8		
	Combined silica (%) with S.E.		
Summer	11·74 ± 0·519	12·46 ± 0·651	12·85 ± 0·565

TABLE 3. *Extent of infestation in relation to sowing date*

Individual treatments: moderately resistant and susceptible types sown at dates indicated: loss of primary shoots (%)

	16 March		6 April		21 April		
25 April	3-leaf		1-leaf		—		
16 May	7-leaf		4–5-leaf		3–4-leaf		
	Spet × Star	Victory	Spet × Star	Victory	Spet × Star	Victory	Sig. diff.
	3·5	16·8	58·6	78·8	90·3	97·6	6·6
	13·3		20·2		7·3		

TABLE 4. *Reduction of varietal difference in extent of infestation owing to placement of additional eggs on the resistant types*

Growth stage at 1st treatment	Plot	Variety, loss of primary shoot (%) Victory	Summer	*d*	d^1
6-leaf	Treated	76·4	38·9	37·5	8·1
	Control	69·7	24·1	45·6	
5-leaf	Treated	87·5	72·5	15·0	26·5
	Control	89·6	48·1	41·5	
4-leaf	Treated	97·7	88·8	8·9	7·6
	Control	98·1	81·6	16·5	
Sowing date					
2 April	Treated	50·0	21·4	28·6	10·7
	Control	69·7	30·4	39·3	
29 April	Treated	86·8	89·3	−2·5	28·4
	Control	92·6	66·7	25·9	

The effect of the treatment was marked, Summer apparently being no more directly resistant than Victory.

(2) 1942. A repetition of the experiment was made at various growth stages. Two plots of Victory and Summer alternating were sown on 24 March, and again on 2 and 11 April, in the field, each plot carrying 60 seeds of each variety; 320 Summer and 305 Victory plants finally survived. All the plants were subject to natural oviposition from late April onwards. Every Summer plant received two or more eggs between 12 and 14 May; the remaining eggs procured between 15 and 18 May were placed on the Summer plants of the two later sown plots, when there was no external sign of attack; the intermediate sowing was the most heavily treated. The data obtained are given in Table 4. The control plots yielded data according to expectation, inasmuch as the difference at the upper limit of attack was much decreased, agreeing therefore with the data shown in Table 3; the additional eggs placed on Summer in the second sowing markedly and significantly reduced the difference between the varieties, namely, from 41·5 to 15·0%, D/E being 2·65. Even at the upper limit of the range of attack the difference was reduced in the same direction. The later sown plots might have shown 100% infestation for the treated Summer plants, but, owing to (1) the difficulty of ascertaining the condition of the eggs used for treatment, and (2) the preference shown by the larvae for tillers in the later growth stages, it is reasonable that complete infestation should not have been achieved. For example, during 1940, egg viability was estimated between 8 and 14 May; 90 eggs, collected daily in small batches to ensure recent deposition, were incubated at 85° F. in a biological incubator, but 40% of the eggs failed to hatch. Further, no marked effect was expected with the earliest sowing, for as shown by the following experiment the larvae normally work into the tillers at the 6-leaf stage. During 1937, larvae capable of travel were placed in various positions on Summer and Victory plants, pot-grown and kept in the town to reduce the extent of natural infestation; control plants were used at each treatment, and these, numbering 28 in all, were all uninfested. In the following synopsis, positive figures indicate the number of times destruction of the growing point occurred, and negative, ineffective treatment.

	Leaf stage			
Site for larva	3	4	5	6
Soil, adjacent	+2	+4	−2	−2 and +5 tillers
Coleoptile	−2	+1	+1	—
1st leaf sheath	—	+2, −2	+1	−5 and +2 tillers
2nd leaf sheath	—	—	—	— and +3 tillers
3rd leaf sheath	+2	+1	—	—
5th leaf sheath	—	—	+2	—

(3) 1943. An attempt was again made to raise the susceptibility of Summer above that of Victory. A similar sowing on 2 April, involving a total of 222 plants, only confirmed that the natural difference could be reduced, but a late sowing made on 29 April, involving a total of 143 plants and not being totally destroyed, showed the full effectiveness of the treatment (see Table 4). A difference of 25·9%, normal for the period, has been converted into a difference of −2·5%. With these small plant numbers, D/E for Summer = 2·46, i.e. the chances of the increase from 66·7 to 89·3% being significant are about 77 to 1.

The most resistant type known, Summer oat, proved to be as susceptible as Victory oat under these conditions, and a reasonable inference is that resistance is indirect and due to plant attractiveness, which induces the fly either to visit certain varieties more frequently or to oviposit more freely thereon.

Alternatively, there is the possibility that direct resistance of considerable variability exists within a variety and that increased oviposition provides increased numbers of larvae capable of overcoming such direct resistance; but, so far, no evidence of the existence of direct resistance has been obtained.

Oviposition and its Relation to Indirect Resistance

In the case of the resistant variety Summer, resistance to attack has been shown to be indirect and apparently dependent on extent of oviposition, which in turn is dependent on variation in plant attractiveness; extent of oviposition should therefore be correlated with extent of resistance. The following series of observations was made to verify this and probe further into the problem of the origin of resistance.

Victory and Summer

1939. Pots containing four plants of either the resistant type Summer or the susceptible type Victory were grouped alternately and exposed in the field to natural oviposition. The plants were in the 2- or early 3-leaf stages during 26–30 May when the eggs deposited were recorded, the following data being collected:

	Victory	Summer
No. of plants	26	26
Eggs on plant	28	5
Eggs in soil	51	13

Victory attracted nearly 80% of the total number of eggs, while a similar percentage of eggs was deposited in the soil cavity immediately adjacent to the plant in each case, indicating that factors other than those relating to soil were affecting oviposition. The assumption that plant attractiveness exists offers a reasonable explanation of the observed variation.

Confirmation was sought by alternating plants of Victory and Summer in the field and recording from the first to the fifth-leaf stage, oviposition from 3 June being shown in Table 5. The ratios of the differences between the means and their standard errors (D/E) were, for row I, 5·65 and for row II, 3·26, both therefore being clearly significant and confirming the preference for Victory in the ratio of 4 to 1. In this case very few eggs, namely, 22 in all and 19 against Victory, were found in the soil. The Summer plants in row I received a mean of 2·2 eggs per plant, from which 100% infestation would have been expected. Reduction to 53% was probably due to highly irregular placement.

1943. The reality of differential oviposition was established beyond doubt. Fifty plants each of Victory and Summer, sown alternately, were observed between 12 and 20 July while passing from the 1-leaf to the 3-leaf stage. From 105 plants and the adjacent soil 1272 eggs were removed; after the first day, when 54 were found, the daily total varied from 120 to 169, averaging 152, their distribution being shown in Table 6. Considering only the oviposition on 46 pairs of adjacent plants and analysing the differences by 'Student's' method, the mean difference of 12 was proved to be significant.

The percentages of eggs found in the soil near Summer, near Victory, and altogether, were 23·4, 26·3, and 25·6 respectively, that is, in proportion to plant attractiveness. Maximum oviposition occurred on 18–19 July in accordance with expectation, but the data quoted below for the abnormal conditions prevailing during the spring of 1945 show that fly prevalence periods may be liable to greater variation than previous observations indicated (Cunliffe, 1921, et seq.). In spite of the intensity of the search only 34 plants escaped attack, showing the difficulty of obtaining complete records.

When the soil was dry 49·5% of the eggs were deposited therein, the percentage dropping to 10·3 in wet soil. The detailed records show that both varieties were approximately equally affected (see Table 6).

1945. All the above data were collected from late sowings to ensure an adequate egg supply for statistical analysis. An attempt to determine the variation under normal spring conditions, using Victory and Summer plants reaching the 1-leaf stage in the middle of April, was defeated because the temperature was abnormally high during this period, resulting in abnormal immigration and heavy oviposition equalling that recorded during summer months; at the end of the month a fall of snow, followed by a wet period, stopped observation. The oviposition data set out in Table 7 were obtained between 16 and 26 April, the plants passing from the 1-leaf to the 4-leaf stage during this period. Statistical analyses showed that the mean differences for exposed stem and blade, coleoptile, and soil were 0·5, 1·5 and 17·0 respectively, all clearly significant. During this dry period, most of the eggs were again placed in the soil, namely, 89·3% for Victory and 89·5% for Summer. Even the differences in the daily oviposition in the soil adjacent to each variety were as clearly significant as in the case of the total differences, although soil conditions were identical for each variety; for example, the lowest mean difference observed during nine daily observations was 2·2, for which x exceeded 0·9999.

The olfactometer data quoted below in the section 'Olfactometer experiments' show that, so far as is known, chemotaxis plays no part in plant attractiveness. A factor influencing site may be hydrotactic or stereotactic; the olfactometer data for Victory foliage and dry air, showing equal reactions, indicate the likelihood of this second factor being stereotactic; wet conditions which reduce soil oviposition may consolidate the soil round the stem and close the gap, thereby tending to increase oviposition on the plant.

Resistance being indirect and oviposition on the susceptible type three times as great as on the resistant type, estimations of infestation resulting from different intensities of oviposition should correspond approximately with actual infestation data obtained in the field. Theoretically, under conditions of high, medium and low oviposition, the differences in percentage infestation of primary shoots of Victory and Summer should be almost extinguished under high and low conditions and at a maximum under medium conditions. Viable eggs are deposited in the ratio of 3 to 1 in favour of the susceptible type, so that, given equal plant populations and ignoring the influence of possible secondary factors, oviposition resulting in 100% infestation of Summer will necessarily produce 100% infestation of Victory; a 30% infestation of Summer will correspond with a 90% infestation of Victory, i.e. the difference is increased to 60%; while a 1% infestation of Summer implies a 3% infestation of Victory, i.e. the difference is again negligible. A degree of non-viability of eggs merely reduces the degree of attack but, within limits, it obviously becomes more important as the risk of attack increases.

In practice, plants can be exposed to such variations in oviposition rates by the choice of suitable sowing dates, as oviposition normally commences about 25 April and reaches a maximum about 15 May, and the type of field experiment set out in Table 3 shows that theoretical and actual differences

TABLE 5. *Oviposition on alternating plants of Victory and Summer*, 1939

	Victory		Summer	
Row ...	I	II	I	II
No. of plants	15	12	15	15
No. of eggs on leaf stage 1	8	—	0	—
2	50	—	10	—
3	80	38	11	11
4	94	42	20	11
5	89	30	23	0
Mean per plant with S.E.	9·4 ± 1·21	4·3 ± 0·91	2·2 ± 0·40	1·2 ± 0·31
Loss of primary shoot (%)	100	100	53	60

TABLE 6. *Distribution of eggs on susceptible and resistant varieties of oats*, 1943

(*a*) According to variety of oat*

	No. of plants	No. of eggs	Max.	Mean	Min.
Victory	53	964	37	18	1
Summer	52	308	13	6	1

(*b*) Site according to soil condition

			Ratio
Dry conditions: Plant	Victory, 186	Summer, 65	2·86
Soil	Victory, 188	Summer, 58	3·24
Wet conditions: Plant	Victory, 524	Summer, 171	3·06
Soil	Victory, 66	Summer, 14	4·72

* Further oviposition observations on extreme types failed to establish any significant differences within a variety.

TABLE 7. *Differential oviposition data for Victory and Summer*, 1945

No. of plants, Victory 48 and Summer 59

		Max.	Mean	Min.
Exposed stem and blade	Victory	4	0·58	0
	Summer	2	0·07	0
Coleoptile	Victory	10	2·4	0
	Summer	6	0·8	0
Soil	Victory	43	24·7	3
	Summer	19	7·6	1

TABLE 8. *Differential oviposition on parent types and selections from crosses, and also wheat and barley*

	Variety	No. of pairs	No. of eggs removed			Ratio
			Max.	Mean	Min.	
(1)	Victory	31	33	11·0	1	2·97
	Summer		10	3·7	0	
	Victory	33	—	10·8	—	1·66
	Star × Summer		19	6·5	1	
	Star × Summer	30	—	5·8	—	1·61
	Summer		—	3·6	—	
(2)	Star	29	49	24·1	9	2·94
	Spet		22	8·2	2	
	Star	34	—	25·7	—	1·65
	Sandford		34	15·6	1	
	Sandford	31	—	15·5	—	1·82
	Spet		—	8·5	—	
(3)	Oats	40	47	20·5	5	1·34
	Barley		29	15·3	5	
	Oats	40	—	20·5	—	2·14
	Wheat		26	9·6	2	
	Barley	40	—	15·3	—	1·59
	Wheat		—	9·6	—	

in infestation are of the same order. Here, using a Spet × Star resistant selection and Victory, Spet × Star being less resistant than Summer, the 16 March sowing showed a difference of 13·3%, the 6 April sowing 20·2% and the 21 April sowing 7·3%, a difference of 6·6 being significant.

It may be concluded, therefore, that among the varieties studied, although the plant itself has no direct resistance at least in the important early stages of growth, varietal differences in resistance occur because of the preference exhibited by the fly for a certain variety for oviposition.

Paired types

1940. An attempt was made to determine the relative power of attraction of the extreme types, Victory and Summer, by recording oviposition on sequences of pairs of plants [sown together—120 in all], during the period 10–21 May, when the plants passed from the 1-leaf to the 3-leaf stage:

Types		Susceptible + susceptible	Susceptible + resistant		Resistant + resistant
Susceptible	Resistant				
Victory	Summer	30	30	1	23
Star	Spet	75	48	12	33

Oviposition was very erratic, resulting in a value for D/E of 2·12 only. The ratio in favour of the susceptible type as a whole was again 3·7 : 1, and the ratio was increased in the case of the mixed groups as a whole, but the spatial conditions brought about by soil obstructions were too irregular for accuracy.

Confirmatory evidence from other varieties and selections from crosses

1944. Obviously, extension of the observations, to include other extreme types and strains from crosses between extreme types all of known history in relation to resistance in the field, was necessary to establish the correlation as a general principle. Oviposition records were obtained from Victory, Star, Summer, Spet and selections from Summer × Star and Spet × Star crosses which in the field have proved to be intermediate in resistance.

Victory, Summer, and Summer × Star were sown in regular sequence in four rows, totalling 120 seeds; examination began when the plants were in the 1-leaf stage and was continued to the early 4-leaf stage, 805 eggs being removed during the period 4–17 July. Statistical analysis was based on adjacent pairs only, hence the minor differences between means for the one variety in the summary of the data shown in Table 8.

Confirmation was obtained by examination of a second set of resistant and susceptible parents and a selection from a cross between them, all of known resistance in the field. Spet, Star and Spet × Star (Sandford) were sown in the same manner as before, and 1765 eggs were collected from this material during the period 15–23 June, the distribution of eggs being given in Table 8. For five of these comparisons, x for the mean differences exceeded 0·998; for Star × Summer/Summer, $x = 0{\cdot}9878$. Egg deposition ratios for comparable pairs in the two groups are very similar; the susceptible types received three times the number of eggs placed on or near the resistant types, while the particular selections from the crosses were almost exactly intermediate.

These observations are in accordance with field-resistance data. Field measurements of resistance for the extreme types and for the Spet × Star selection have been published (Cunliffe, 1936), and ample confirmatory evidence has accumulated since 1935 during selection trials. Data have been abstracted from several Latin square trials to show that Sandford, a selection from a cross between the extreme types Spet and Star, is consistently intermediate in resistance. Victory and Star are equally susceptible and Summer and Spet are equally resistant, but Spet and Star have not been widely used as standards. It has been pointed out above that differences at the extremes of the possible range, although generally significant, are necessarily small and not typical of normal conditions; therefore weighted mean differences have been calculated from data accumulated over the years 1936–44, including only those cases in which Victory records ranged from 50 to 95% infestation of primary shoot:

Comparison	% difference	E	D/E
Victory, Summer	42·2	1·54	27·4
Victory, Spet × Star (Sandford)	32·8	1·54	21·3

Only one direct Latin square comparison between the Spet × Star and Summer × Star selections has been made, namely, during 1944, when the percentage losses of primary shoots were 37·1 and 44·8 respectively, with a significant difference of 8·5 and a Z value of 1·700 (0·7058 for 1% point of distribution), i.e. they are equally resistant.

Oviposition and resistance are positively correlated in the case of these varieties.

Other cereals compared with oats

Oats, wheat and barley differ in degree of infestation, a late sowing on 29 April 1943 of 150 seeds of the three cereals in sequence giving the following

result on examination when in the 5-leaf stage on 5 June:

	Loss of primary shoot, % with S.E.
Wheat, dark green	59·6 ± 7·6
Barley, very light green	61·9 ± 7·1
Victory oats	97·8 ± 2·2

The greater susceptibility of oats is clearly significant; therefore, if this order of attack is typical of the three cereals, oats should receive the largest number of eggs. During 1944 oviposition was recorded on Squarehead's Master (white chaffed) wheat, Cambridge 7/11/1 barley (Praecox × Spratt-Archer) and Victory oats, the original varieties of wheat and barley used not being obtainable. The seeding arrangement was the same as before; 1817 eggs were removed from the plants during the period 24 July to 1 August, the plants passing from the middle 1-leaf to the middle 3-leaf stage during this period, the data being shown in Table 8. No obvious colour differences between the plants were apparent to the eye, unlike the previous case, yet the records show clearly significant (x exceeding 0·999 for each comparison) differences in oviposition; proportional oviposition was 4, 3 and 2, for oats, barley and wheat respectively, for these particular varieties. During 1945 these same varieties of oats, barley and wheat were examined for extent of infestation under field conditions. They were included with Victory, Sandford and Aberystwyth S 175 in a Latin square sown on 26 March, giving the following data: for the individual varieties, the percentage losses of primary shoots for Victory, S 175, Sandford, Sandford, wheat and barley were 98·4, 94·5, 88·6, 88·2, 77·5 and 46·3 respectively, the significant difference being 7·4. Wheat may be showing the full extent of possible infestation under these conditions, Victory being too near the upper limit, hence infestation and oviposition may be correlated, but this is certainly not so in the case of this variety of barley, in which the discrepancy may be due to direct resistance or earlier tillering capacity.

Reverting to the 1944 series, the total distribution of eggs was as follows:

	Blade plus stem	Coleoptile	Soil	Total
Oats	13	231	577	821
Wheat	8	57	318	383
Barley	17	368	228	613

During the recording the impression was that the flies were placing more eggs, in proportion, in the barley coleoptile than in the oat coleoptile, and although the variability was great, statistical analysis showed that this was probably true. Comparison of 40 pairs of oat and barley plants indicated a mean difference of three eggs in the coleoptiles, x being 0·9762 for 30 readings, i.e. the chances of the difference being significant are 41 to 1 for 30 readings, while actually there were 40. Wheat received very few eggs in the coleoptile, the great majority being placed in the soil.

These coleoptile records were suggestive, because it had also been observed that the barley coleoptile separated from the stem before those of either oats or wheat, and it was possible therefore that this was the reason why barley received so many eggs in the coleoptile and so few in the soil. Oats received the larger total of eggs; if plant attractiveness alone had operated, the data would presumably have been comparable with those of the former series, which suggested that plant attractiveness determined the total number of flies, while another factor determined the site of deposition, and the expected distribution would have been somewhat as follows:

Site of eggs	Coleoptile + soil	Coleoptile	Soil
Oats	808	231	577
Barley	596	170	426

the barley 'soil eggs' then being 74% instead of 39% as actually occurred.

In this series, however, soil conditions were similar for all, yet the 'soil egg' ratios were very different from the total ratios. The 'soil egg' means for oats and barley show a difference of 349 for 40 pairs, giving a mean difference of 9 which is clearly significant.

The influence of another factor is indicated, and it is possible that this factor is coleoptile attractiveness, due to a stereotactic reaction.

Wheat received 85% of its eggs in the soil and would therefore be expected to have a coleoptile showing longer adherence. Hairiness of wheat cannot explain the difference, as the oats and barley, both glabrous, show significant differences.

Influence of coleoptile condition

The next question is whether preference is in fact partly determined by the growth variations of the coleoptile. During August 1944, an egg count was made to determine the effect, if any, on the extent of oviposition, of separating the coleoptile from the stem at the earliest stage. The separation was effected on alternate plants of rows of Victory oats on 10 August, when the plants were in the early 1-leaf stage. Treated stems tended to return to normal, and the separation had to be repeated in many cases after the day's count as it was considered undesirable to introduce foreign holding substances.

During these observations the soil was very dry and no rain fell during the period 11–18 August; rain from 19 August onwards stopped observation. From two rows, each originally of 30 seeds, 2297 eggs were removed; the data from adjacent pairs of

plants, involving 2037 eggs, are summarized in Table 9. The significance of the treatment is clear; the act of separation of the coleoptile from the stem doubled the attractiveness of the plant over a period of 7 days. The daily differences were pronounced and clearly significant until the control naturally assumed the same condition as the treated type, when significant differences ceased. Analysis of the data, excluding the coleoptile eggs, showed that the effect of the treatment was due mainly to the coleoptile differences; there was a mean difference of eight eggs in favour of the treated plants, but the chances of this difference being real are only 35 to 1. The percentage of eggs deposited on the treated plants on stem plus blade and in the soil was 56·8 for each site, whereas the percentage in the coleoptile of the treated plants was 90·8.

The correlation between the differences in the percentages of the daily totals of eggs placed on the coleoptile and in the soil for the control and treated series is $r = -0{\cdot}825 \pm 0{\cdot}075$; thus, although the separated coleoptile doubled the oviposition, the percentage coleoptile gain is balanced by the percentage soil loss.

A repetition of this experiment, made during 1945, using the resistant variety, Summer, was completely confirmatory, as shown by the data presented in Table 9. In this case the coleoptiles were separated on 29 June, when the plants were 1 in. high.

Every effort was made to avoid tearing the tissues, to eliminate undetected chemotactic reactions, but some tearing was inevitable. Possibly the control tissue should have been bruised also, but oviposition preference is apparently, under normal conditions, not due to chemotactic reaction.

Observations made during 1945 in the field showed that substantial bruising of Victory oat tissue failed to have any effect on extent of oviposition; eggs on 27 bruised plants averaged 5·8 per plant, as against 5·6 for 27 alternating controls.

On the other hand, when the resistant type Summer was smeared with sap from the susceptible type Victory, it was found that oviposition was markedly affected. Rows of 30 Summer seeds were sown during August 1945, and alternate plants were smeared with sap pressed from Victory plants on 3 and 4 August, the controls being wetted at the same time. One count only was possible owing to inclement weather, 236 eggs being removed from 28 plants and the adjacent soil on 6 August. The control plants averaged 10·8 eggs and the treated plants 6·1 eggs per plant, the difference of 4·7 being clearly significant. A mean difference of 3·6 in the numbers of eggs placed in the soil adjacent to control and treated plants was also significant, in favour of the control plants. Any deterrent effect due to decomposition products should also have been produced by bruising; thus although the position is not yet clarified, damage to tissue evidently did not increase extent of oviposition.

It would seem then that two factors were operating to determine extent of oviposition, namely (1) plant attractiveness, equal for control and treated plants and resulting in equal percentage deposition of eggs, for each type, in coleoptile and in the soil, the ratio probably being determined by a stereotactic factor, namely, the extent of soil consolidation, and (2) coleoptile attractiveness, a definite stereotactic factor, resulting in increased deposition on plants with open coleoptiles and a change in the coleoptile/soil ratio.

It remained to determine how this question of coleoptile condition might be affecting differential oviposition on Victory and Summer oats, which show a significant difference in coleoptile oviposition as indicated in Table 7 and also by re-examination of the data shown in Table 8, as set out in Table 11. In the case of the coleoptile eggs, all the differences are significant at the 1 % point of distribution except for Victory/Star × Summer, for which comparison the chances have dropped to about 50 : 1 for 35 observations. Except for the comparison Star × Summer/Summer, where the numbers of eggs are small, the differences between the soil eggs are significant and of the same order as in the coleoptile series.

Changes in coleoptile condition during growth were observed in the case of Victory and Summer oats using 300 plants of each, sown in March 1945. Practically no varietal difference existed; in both varieties, separation was spread over 20 days or so after appearance above ground (10% being very late and erratic). For Victory, the mean percentage daily increase in the numbers separating was of the order of 7·5 %; the minimum and maximum percentage daily increases were 2 and 12 respectively; up to the 3-leaf stage Victory was slightly in advance of Summer, by about 3 %, and subsequently consistently later to the same extent.

Differential oviposition in this case of extreme types cannot therefore be ascribed to the influence of the coleoptile and must be ascribed to differential plant attractiveness.

Interpretation of the oat, wheat and barley data on these assumptions does offer a reasonable explanation of the apparent discrepancy in the barley data. Assuming the operation of the two factors in the manner indicated and a plant/soil ratio of 1 : 3, as observed for oats and wheat, the percentage distributions to the nearest whole number, according to observation and expectation, are indicated in Table 10, and it will be noted that they are in close accord, thereby providing confirmatory evidence of the existence of the two factors. The relative values of plant and coleoptile attractiveness naturally would not necessarily be so clear-cut as indicated in

TABLE 9. *Effect of coleoptile on oviposition*

(1) Distribution of eggs

	Exposed stem and blade	Coleoptile	Soil	Total
Victory control	64	58	545	667
Victory treated	84	573	713	1370
Summer control	34	8	63	105
Summer treated	31	80	92	203

	No. of pairs	No. of eggs removed: Max.	Mean	Min.	Sig. diff.
(2) Total no. of eggs					
Victory control	23	52	29·2	13	3·0
Victory treated		82	59·8	30	
Summer control	24	11	4·3	1	4·1
Summer treated		15	8·5	3	
(3) (*a*) Total no. of eggs less no. inside coleoptile					
Victory control	23	52	26·3	11	8, not sig.
Victory treated		62	34·8	13	
(3) (*b*) No. of eggs on coleoptile					
Summer control	24	4	0·3	0	3·0
Summer treated		8	3·3	0	

TABLE 10. *Explanation of the apparent discrepancy in the barley oviposition data*

	Oats	Wheat	Barley			
Proportional oviposition due to:						
Total attractiveness	4	2	3 by observation			
Plant attractiveness	4	2	1½ assumed, from Victory data			
Coleoptile attractiveness	0	0	1½ do.			
	Soil	Plant	Soil	Plant	Soil	Plant
Percentage of total eggs:						
(*a*) By observation	32	13	18	3	13	21
(*b*) By allocation in the following way:						
Plant attractiveness	34	11	16	5	13	4
Coleoptile attractiveness	—	—	—	—	—	17

TABLE 11. *Detailed analysis of the oat data presented in Table* 8

(*a*) Location of eggs

	Stem and blade	Coleoptile	Soil
Victory	46	226	123
Summer	17	60	53
Star × Summer	31	134	62
Star	16	371	501
Spet	4	91	195
Sandford	6	233	348

Comparisons	No. of pairs	Means	Mean diff.
(*b*) Coleoptile eggs			
Victory/Summer	29	5·7 : 1·5	4
Victory/Star × Summer	35	6·1 : 3·8	2
Star × Summer/Summer	29	3·4 : 1·5	2
Star/Spet	29	10·2 : 2·5	8
Star/Sandford	35	10·6 : 6·4	4
Sandford/Spet	31	6·2 : 2·6	4
(*c*) Soil eggs			
Victory/Summer	29	3·3 : 1·5	1·8
Victory/Star × Summer	35	3·4 : 1·7	1·7
Star × Summer/Summer	29	1·6 : 1·4	0·2
Star/Spet	29	13·4 : 5·6	7·8
Star/Sandford	35	14·3 : 9·4	4·9
Sandford/Spet	31	9·1 : 5·7	3·4

the above example, in which possible disturbances, e.g. by wheat coleoptile attractiveness, have been ignored. The two factors may act in opposition, as in the case of these particular varieties of barley and oats.

ORIENTATION OF THE FLY

No evidence has been obtained to indicate that oviposition is in any way indiscriminate; so far, in the field, attempts to induce oviposition on artificial media have been unsuccessful and eggs have never been found away from the plant. Two questions arise, namely (1) what attracts the ovipositing female to the Gramineae, and (2) what causes it to differentiate between types? The answer to the first question would no doubt provide a clue to the answer to the second, but no line of approach to the more general problem is suggested by available data. Investigation within the more limited field has been concurrent with the oviposition studies; the results so far have been negative regarding the origin of plant attractiveness, although they are of general interest.

Olfactometer experiments

Orientation induced by chemotactic reaction suggests itself as offering the simplest explanation of differential oviposition, but olfactometer experiments gave negative results.

During 1940, flies were bred in the laboratory, offered a nutrient solution of sugar, and their response to stimulation tested in an olfactometer, no fly being used twice; the apparatus was generally run for 1 hr. before examination. With light only, the reaction was equal in both limbs, 56 and 59 flies responding respectively, the sexes being attracted equally. The following data are typical of trials with both varieties. With crushed Victory foliage in one limb and moisture only in the other, and the materials then reversed, the reaction was 26 and 37 flies respectively. With crushed Victory foliage against dried air, followed by reversal in the limbs, the reaction was 158 and 132 respectively.

With crushed Victory plants in one limb and crushed Summer plants in the other, followed by reversal in the limbs, the reaction was 53 and 65 flies respectively.

Finally, water and ether extracts from the two varieties failed to show any differentiation.

Thus chemotaxis fails to offer any explanation of differential oviposition, unless it is inhibited by phototaxis, under these conditions.

Orientation to light

With oats, it has been shown that orientation is not due to either chemotaxis or hydrotaxis; stereotaxis appears to exert some influence, as exemplified by the effect of the separation of the coleoptile, but, as deposition in the soil shows, does not offer a complete explanation. The most resistant types of oats, such as Summer, reflect much less white light to the human eye in the more susceptible early growth stages than Victory does, and it seemed reasonable, as a starting point, to investigate the possibility of the reaction being governed by phototaxis. Caution is required when making inferences from phototactic reaction data, because, in the first place, insect and human reactions are not necessarily equivalent and, in the second place, total energy of radiation as well as spectral region may be involved when determining extent of reaction using white light as a standard.

The effective difference in the radiation reflected from the two plant surfaces may be quantitative, qualitative or a combination. If quantitative only, then the fly may react positively to the plant reflecting the greater total energy, colour being ignored; the darker Summer, reflecting less, would appear less bright to the fly. If qualitative, the effective spectral region may or may not be beyond human vision; reaction may be due to colour differences as seen by the fly, ultra-violet possibly appearing as a colour to the insect. In each case oviposition would be proportional to attractiveness. Measurement of reflected radiation involves the use of an integrating sphere and a photoelectric lumen photometer, a specialized unit not now available, for determining the differences between the plant types in regard to reflexion. Such studies might indicate the factors involved in the primary attractiveness of the Gramineae, in the absence of which it is difficult to understand how a comparatively simple phototactic reaction can operate. Suitable variations in intensity and colour must occur in all directions unless, for example, reaction is governed by some such factor as the ratio between reflexion of certain spectral regions.

Thus the preliminary study was necessarily confined to observation of the effect on the orientation of the fly of (1) varying intensities of white light, and (2) particular regions of the spectrum.

Variation in intensity of white light

A few experiments were made with paper screens and the apparatus described below which showed that the fly was responsive to variations in intensity of white light, but no attempt was made to collect data for statistical analysis as neutral density filters could not be obtained. The small range of experiments did indicate that the reaction was regular when the illumination of the control light was of the order of 90 f.c.; approximately, a reduction of illumination to 15 % reduced the reaction from 50 to 5 %, whereas a reduction to 75 % reduced the reaction from 50 to 35 %. So a reduction to about 30 % should give a reaction of 25 %, sufficient to explain the 1 : 3

oviposition ratio. Actually illumination reductions of approximately 32, 40 and 45 % reduced reactions from 50 % to 30, 22 and 25 % respectively in three tests. These data do not necessarily indicate that the same order of result would be obtained using reflected daylight from the two types of plant surface; further, it is possible that the effect may be due to the reduction in intensity of some particular region of the spectrum. More precise observation had to be deferred, pending the supply of neutral density and regional filters. Although this reaction might explain the differential oviposition on alternating Victory and Summer plants, it fails to account for consistent differences between large oat populations and also the low figure for the wheat variety, Squarehead's Master, which has much the same colour as oats. Further, reaction to intensity of reflexion of white light alone seems doubtful, owing to the great variation in intensity of daylight and between plants of all types.

Orientation to spectral regions

Thus it seemed more likely that reaction to spectral region might lead to an explanation of the facts, although, again, the absence of indiscriminate oviposition seemed to imply the operation of other factors also.

Numerous experiments with various coloured materials compared with white light and amongst themselves, showed that the phototactic reaction of the fly was normal, inasmuch as radiation covering the spectrum from green to red was of low attractive power.

Eggs are set mainly either inside the tip of the coleoptile immediately after it has begun to separate from the stem or in adjacent soil. To the human eye, in addition to the difference in foliage colour, there is a very obvious difference in colour between the stems at this point, Victory being very pale green and Summer purple. Normally, the purple coloration of Summer extends to the base of the first leaf sheath, but at lower temperatures it may include the exposed part of the second leaf sheath also. The stem of Victory would be expected to have strong absorption bands in the blue region and that of Summer in the green-yellow region; if the visible region were effective, then Victory should appear less bright to the fly and be less attractive. But the opposite is the case, which suggests reaction to the non-visible region. Much of the pigment of Summer is soluble in water and is probably due to the development of anthocyanin, which has absorption bands in the ultra-violet region.

According to Duggar (1936), very little is known about plant reflexion below 4100 A. Seybold, quoted by Duggar, found that from green leaf sections reflexion in the regions of 3360 and of 4360 A. averaged about 10 % of the incident light, whereas from white leaf sections the percentages were 18 and 27 respectively. Thus one might expect even more difference between reflexion from the very light green Victory stem and from the Summer stem coloured by anthocyanin.

As reaction to the longer wave-lengths is small, three filter solutions were used to cut off parts of the spectrum below 3800, 4100 and 4800 A., respectively, to obtain a broad idea as to which region might be most effective in producing a reaction, neglecting the question of intensity until this preliminary information had been secured, but also making use of a mercury-vapour lamp to secure an intensification of ultra-violet radiation for comparison with daylight. Ultra-violet is cut off at 3800 A., ultra-violet and most of the violet at 4100 A. and the two latter plus the blue at 4800 A.

The three filter solutions used were: (*a*) for the 3800 A. limit, 1 cm. thickness of quinine hydrochloride in water, 2 % solution; (*b*) for the 4100 A. limit, sodium nitrite, half-saturated; and (*c*) for the 4800 A. limit, picric acid, one-quarter saturated. The use of quinine hydrochloride was somewhat difficult with limited supplies, as it undergoes immediate and continuous oxidation on exposure to light, due to photo-chemical action; a deepening of the original yellowish tinge makes it approach sodium nitrite in action. It is an excellent filter for cutting off ultra-violet only, because it cuts sharply.

The simplest of apparatus was used, namely, a lidded light-tight wooden box, about 1 ft. cube, with two holes of $1\frac{1}{4}$ in. diameter on one side spaced at $4\frac{1}{2}$ in. between centres. Into each hole was inserted a double tube about 9 in. long with vent for emptying, the spacing between the inner and outer tubes being 1 cm. Flies bred from attacked stems or waste from a combine harvester were sorted by sex and quickness of reaction to daylight, only those females reacting within 2 min. being used in most cases. The apparatus was normally left for about 1 hr. when used in bright sunlight, and for 2 hr. when used with a mercury-vapour lamp. Latterly, the tubes were arranged at right angles to one another, so that the beams met at a place about 4 in. in front of the fly release point.

The balance of the apparatus was fair; with distilled water in each side typical readings were of the order 44–56 and 48–52 %, but occasionally there was no response at all in half an hour, probably due to treatment. Regular alternation of tubes and solutions failed to reveal any bias due to tube or position. The data obtained are summarized in Table 12.

The reactions of the sexes, under the same experimental conditions, appeared to be similar, according to data derived from material sexed after experiment. In five of the experiments, mean reactions for males and females were 23 and 21 %

respectively. The effect of various disturbing factors, such as uneven illumination at tube entrance, physiological condition of insect, etc., is clearly shown in the large standard error in the picric acid series, so probably the picric acid/mercury-vapour lamp result has no significance. As the data obtained from the mercury-lamp trials otherwise resemble those obtained from daylight trials, these few experiments suggest that the reaction is due to ultra-violet radiation alone, and that no increase in ultra-violet radiation over that present in sunlight produces any increase in reaction. The question of the effect of variation of total energy of radiation produced by variation of energy in the spectral region under examination has been ignored for the moment. Some confirmation was obtained by comparing reactions when using one or other substance as a standard. As was to be expected from the results of comparisons with distilled water which showed that no further reaction resulted from the abstraction of wave-lengths above 3800 A., no marked differences were observed. Quinine hydrochloride against picric acid gave reactions of 48 and 32 flies and against sodium nitrite 64 and 54 flies respectively.

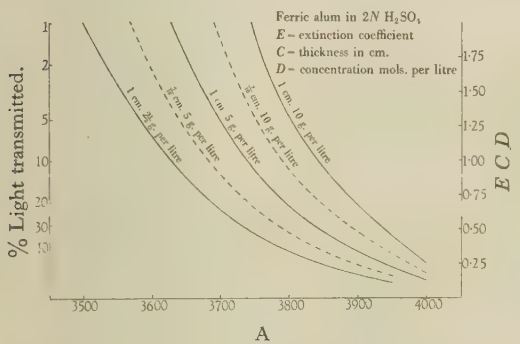

Fig. 1. Percentage of light transmitted by ferric alum filters.

No effect resulted from cutting off red and heat rays by using 20% $CuSO_4$, with distilled water as a standard; the filtered light attracted 47 flies as against 52 for the standard.

Although the fly showed such a marked reaction to ultra-violet radiation, when eventually it became possible to photograph Summer and Victory in the light of an iron arc, using a screen passing ultra-violet light only and a quartz lens, no obvious difference in negative density was apparent.

As it was possible that non-correlation might be due to the use of the whole of the ultra-violet region, other filters were sought to enable finer cuts to be made within this particular region. It is apparently difficult to find simple but effective filters for the purpose of cutting off different parts of the spectrum below 3800 A., but eventually ferric alum in 2*N* H_2SO_4 at the rate of 10 g./l. was found to cut at 3700 A., diluted solutions cutting at lower wave-lengths.

The percentages of light of the various wave-lengths transmitted by ferric alum filters of different strengths and $\frac{7}{10}$ cm. thickness are shown in the graph in Fig. 1 approximately only, full data as to the relationship between $E \times C \times D$ and percentage transmission not being available. From this it will be seen that a 10 g./l. filter cuts off at about 3700 A., a 5 g./l. filter at about 3575 A. and a $2\frac{1}{2}$ g./l. filter well down towards 3400 A. Measurement of the absorption of the 10 g./l. filter, using distilled water as a standard, proved that very little absorption occurred above 4050 A.:

Wave-length (A.)	Optical density	Absorption %
6400	0·087	2·5
6000	0·087	2·5
5400	0·087	2·5
5000	0·087	2·5
4360	0·087	2·5
4050	1·227	13·3

Now the glass traps used cut off at about 3150 A., so the fly reaction is governed by the transmission between 3150 and 3800 A.

Using the same apparatus the data shown in Table 13 were obtained.

The steepness of the spectrophotometric absorption curve determines the effective action of the filter; filters rarely pass equivalent intensities at the different regions of the spectrum, so identical reactions by the fly cannot be expected even when they cut completely at the same point. The use of ferric alum in 2*N* H_2SO_4 as a filter solution was almost as effective as the use of quinine hydrochloride. Ferric alum, 10 g./l., cut at 3700 A. and ferric alum, 5 g./l., only transmitted about 14% at 3700 A., and both, when compared with distilled water, had a marked effect on the reaction of the fly, only 25·3 and 29·2% respectively being attracted by the light deficient in ultra-violet. The difference between the two means is not significant, D/E being only 1·4.

Using ferric alum, $2\frac{1}{2}$ g./l., which, by deduction from the graph, would appear to cut at about 3450 A., i.e. above the limit for glass (about 3150 A.), very variable results were obtained, perhaps due to the varying intensity of ultra-violet caused by varying cloud cover; consequently, although a reduction in the mean difference occurred, it has not been proved that a real difference exists, the chances of significance being only 40 to 1. It would appear therefore that the effective region of the ultra-violet range lies between 3400 and 3700 A. But again, photography by the light of a screened mercury-vapour lamp (transmitting ultra-violet light only, mainly 3660 A.) failed to distinguish between the foliage of the two types; the coloured stem of Summer actually gave a somewhat denser negative than Victory.

The inference from these data is that ultra-violet radiation reflected from the stem is not the factor determining plant attractiveness. These various cereals were examined in monochromatic radiation, derived from a mercury-vapour lamp screened by suitable combinations of filters as recommended by Bowen (1942), namely, for yellow, 5780 A. ($A+B$, 1 cm. and C, 2 cm.), for green, 5460 A. ($A+B$, 1 cm. and D, 1 cm.), for blue, 4360 A. (H, 2 cm. and K, 1 cm.) and for violet, 4050 A. (H, 2 cm. and I, 1 cm.). In all radiations, except red (7000 A.), Summer reflected less than Victory from the stem, but the blades were of equal brightness except in the very early growth stages. The three particular varieties of oats, wheat and barley used for oviposition counts showed that in monochromatic yellow and green radiations, stems and foliage were of the same brightness; in blue and violet radiation, the effect varied with the sample, with some there being no difference and with others the order of brightness being barley, oats and wheat. The same variation with sample occurred when the plants were photographed in the light of a mercury-vapour lamp on a process plate. As brightness in blue and violet

TABLE 12. *Phototactic reactions of the frit fly*

	No. of exps. made	Total no. of female flies used	Mean % reaction compared with distilled water
(*a*) Light source—daylight			
Picric acid	11	451	18·3 ± 4·05
Sodium nitrite	4	222	18·4
Quinine hydrochloride	3	103	14·4
(*b*) Light source—mercury-vapour lamp (about 10 times ultra-violet radiation of daylight)			
Picric acid	2	99	3·7
Sodium nitrite	5	138	16·6
Quinine hydrochloride	2	67	12·2

TABLE 13. *Phototactic reaction of the frit fly between* 3700 *and* 3150 A.

		Mean % of flies reacting		
No. of exps.	No. of flies used	Ferric alum (10 g./l.)	Distilled water	Sig. diff.
4	365 (females only)	23·0	76·9	53·9
6	2292	25·3 Ferric alum (5 g./l.)	74·6 Distilled water	49·3
4	947 (females only)	29·8	70·1	40·3
4	1435	29·2 Ferric alum (2½ g./l.)	70·8 Distilled water	41·6
5	2342	35·1 Ferric alum (10 g./l.)	64·9 Picric acid ¼ sat.	29·8 not sig.
2	1090	62·9 Ferric alum (10 g./l.)	37·1 Sodium nitrite ½ sat.	—
1	64	53·1 Ferric alum (5 g./l.)	46·9 sodium nitrite ½ sat.	—
2	1021	55·1 Ferric alum (5 g./l.)	44·9 2 % quinine hydrochloride	—
1	441	45·5	54·5	—

radiation even where it occurs is not correlated with oviposition, it fails to suggest an explanation of the origin of plant attractiveness.

RESISTANCE OF OAT VARIETIES TO ATTACK

The search for types of oats possessing greater resistance than Summer is continuous, particularly among 'land' varieties and selections therefrom, received from abroad; so far, none has been found. Known varieties of varying commercial status have also been tested in Latin squares against resistant types during recent years when spatial conditions permitted, the standard normally used being the relative resistance is attainable. Weighted mean differences have been calculated in certain cases, but obviously the trend indicated is of more importance than the actual value recorded.

For the ten varieties included in Table 14, the figure shown for infestation indicates the percentage loss of primary shoots. Only Eagle approaches Sandford in resistance; Eagle is derived from the cross Victory × von Lochows Yellow, the latter being a resistant type from a German 'land' variety. Royal Scot, from Victory × Record, is highly susceptible as would be expected from the frequency with which Abundance, a highly susceptible type,

TABLE 14. *Comparison of varieties of oats for resistance*

Variety	Infestation (%)	Standard	Infestation (%)	Sig. diff.
Eagle	67·0	Sandford	61·0	9·7
Eagle	18·8	Sandford	14·3	8·9
Eagle	49·7	Sandford	33·3	7·6
Royal Scot	31·1	Victory	31·5	8·0
Royal Scot	56·4	Victory	49·9	—
Royal Scot	67·8	Victory	75·4	9·3
Royal Scot	67·8	Sandford	37·3	9·3
Royal Scot	30·2	Sandford	14·3	8·9
Golden Rain II	80·5	Sandford	37·1	8·5
Golden Rain II	66·7	Sandford	33·3	7·6
Golden Rain II	52·9	Victory	56·5	7·2
Resistance	35·9	Sandford	14·3	8·9
Resistance	78·8	Sandford	33·3	7·6
Abundance	59·7	Summer	16·0	7·2
Abundance	81·1	Summer	41·9	9·4
Supreme	71·7	Sandford	37·3	9·3
Marvellous	59·1	Sandford	33·3	7·6
Vestergaard	44·5	Sandford	14·3	8·9
A. ludoviciana	78·8	Sandford	37·3	9·3
Aberystwyth S 175	56·9	Sandford	37·3	9·3

Varieties compared	Weighted mean diff.	*D*/*E*
Eagle/Sandford	+ 9·97	3·99
Royal Scot/Victory	+ 1·08	0·41
Royal Scot/Sandford	+22·90	7·13
Golden Rain II/Sandford	+38·0	13·4
Resistance/Sandford	+35·5	12·5
Abundance/Summer	+42·1	14·7

Spet × Star selection, Sandford. Frequently it is convenient to use one of these commercial varieties to complete a square, necessitating comparison with standards other than the above. It follows that presentation of the data from the squares in the normal way would involve the inclusion of much data concerning selections which are only of temporary interest, and in such cases the relevant data only have been tabulated.

It is not possible from a few trials to arrive at a precise measure of the difference between varieties, under varied conditions of attack, owing to the contraction of the difference as the extent of attack approaches a limit of the range, but an indication of occurs in the production of Record. Golden Rain II, from Victory × Golden Rain, both of which are Milton selections, is again naturally highly susceptible. No data are available relating to the resistance of Grey Winter and Argentine, the parents of Resistance, nor for the varieties White August and White Swedish, the parents of Abundance.

Table 15 includes the comparison of Binder, Mansholt III, Onward and Sun with Victory and Sandford, together with weighted mean differences compared with Victory. All the latter differences are significant except for Onward, which is therefore as susceptible as Victory. Mansholt III is slightly more susceptible, while Binder and Sun are some-

what less susceptible, but markedly more so than Sandford. Sun, produced by backcrossing Eagle with Victory, has less resistance than Eagle. There is no information regarding the resistance of the parents of the other three varieties.

Aberystwyth S 84, Yielder, Richland Iowa 411, Radnorshire Sprig and Black Tartar are compared with Sandford in Table 15. All these varieties except Richland Iowa 411 are highly susceptible; Black Tartar is a selection from Great Mogul, which showed some resistance when compared with Victory in Sweden (Cunliffe, 1929); the parents of S 84 namely, Victory and Red Algerian, are both more susceptible than Sandford; there is no information regarding the resistance of the parents of Yielder and Radnorshire Sprig nor that of Kherson, the Russian line from which Iowa was selected.

Single observations on eight named varieties are set out in Table 15. They are clearly all significantly more susceptible than Sandford; there is no information as to their parentage.

The general inference from these and previous investigations (Cunliffe, 1936), is that resistance is inherited and that three classes are distinguishable among the varieties studied, namely:

(1) Resistant — Summer, Spet and Hede.
(2) Moderately resistant — Sandford, Richland Iowa 411 and Eagle.
(3) Susceptible — In varied degree, all other varieties so far tested.

Grain infestation and sterility

Very little information is available regarding varietal differences in extent of either grain infestation or sterility. During 1940, Victory, Summer, two selections from Spet × Star (one being Sandford) and two selections from Summer × Star were set out on 28 March in a Latin square at the rate of 30 seeds per plot, the panicles being labelled daily as they appeared, to obtain a series exposed to the same conditions of infestation so far as this could be arranged under field conditions. The total numbers of panicles obtained from each selection were Victory 89, Summer 431, Spet × Star (Sandford) 202, Spet × Star (2) 178, Summer × Star (1) 297 and Summer × Star (2) 230, Victory obviously suffering heavily from stem infestation. No one day's production provided a complete series of panicles, and only twice was it possible to obtain complete series, namely, from panicles emerging on 18–19 June and 24–25 June. The panicles produced during these periods were examined for extent of seed attack and sterility, main and bosom grains being kept separate; samples of the total production for each strain were also examined. So far as possible careful distinction was made between insect damage and sterility, but classification was admittedly sometimes difficult.

The size of the sample examined depended on the panicle production, the maximum, mean and minimum numbers of grains examined being given in Table 16.

Table 15. *Comparison of varieties of oats for resistance*

Individual varieties: loss of primary shoots (%)

	Sandford	Victory	Binder	Mansholt III	Onward	Sun	Sig. diff.
1939	22·5	60·5	45·9	55·1	47·7	36·2	10·8
1940	15·4	32·4	35·1	48·0	43·1	25·5	7·1
1941	20·0	56·2	45·4	56·9	60·7	47·6	7·1

	Aberystwyth S84	Yielder	Richland Iowa 411	Radnorshire Sprig	Black Tartar	Sandford	Sig. diff.
1943	63·5	64·1	35·1	60·5	63·9	25·1	8·7
1944	78·7	87·7	51·5	81·8	92·8	47·9	6·2

Tyrone Tawny	Victory	Sixty Day Sel. of Eth.	CA 1/13/6/3 (Cambridge)	Fulghum	Sandford	Sig. diff.
94·1	85·6	85·5	83·0	82·6	65·0	8·0

Green Russian	North Finnish	Early Joanette	Awnless Probsteier	Old Island Black	Sandford	Sig. diff.
88·2	83·5	81·8	80·8	82·9	41·1	6·4

Variety	Weighted mean diff. c/f Victory	*D*/*E*	Variety	Weighted mean diff. c/f Sandford	*D*/*E*
Sandford	−28·8	12·60	Black Tartar	+42·9	17·0
Sun	−10·7	4·72	Yielder	+39·5	15·6
Binder	− 5·9	2·62	Radnorshire Sprig	+34·4	13·6
Onward	+ 4·0	1·75	Aberystwyth S84	+33·4	13·4
Mansholt III	+ 5·8	2·56	Richland Iowa 411	+ 5·8	2·3

No significant variation in seed infestation (Table 16(*b*)) was observed in this particular range of varieties and selections, although it included Victory and Summer which are so markedly different in behaviour during the spring attack. The possibility of minor variation is indicated by data shown in Table 16 ((*b*) (3), Total).

two Spet × Star selections give very similar results, no. 2 being a single plant selection from Sandford made in 1934, while the Summer × Star selections, showing a significant difference of 7·3 %, arose from different F_2 plants derived from one cross. These data indicate that susceptibility to sterility may be inherited. If Star carries sterility of the same order

TABLE 16. *Grain infestation and sterility*

(*a*) Size of samples

Sample	Maximum	Mean	Minimum
June 18–19	250	117	52
June 24–25	222	94	22
June 7–27	266	176	83

(*b*) Percentage seed infestation

	Victory	Summer	Spet × Star (Sandford)	Spet × Star (2)	Summer × Star (1)	Summer × Star (2)	Mean	Sig. diff.	At point of distribution (%)
(1) Samples from panicles which emerged on 18–19 June									
Main	27·6	32·1	27·1	28·8	24·0	18·9	26·4	Nil	—
Bosom	13·9	12·1	13·8	13·0	14·3	10·2	12·9	Nil	—
Total	20·3	20·4	19·6	20·3	18·2	14·5	18·9	Nil	—
(2) Samples from panicles which emerged on 24–25 June									
Main	30·7	37·9	44·9	35·9	38·8	31·0	36·5	Nil	—
Bosom	14·9	24·5	25·1	21·6	19·3	15·6	20·1	Nil	—
Total	22·4	30·8	35·0	28·2	27·3	23·3	27·8	Nil	—
(3) Samples from all panicles which emerged between 7–27 June									
Main	34·6	27·4	34·8	30·9	28·9	28·7	30·9	Nil	—
Bosom	17·6	14·8	18·9	15·8	12·3	16·6	16·0	Nil	—
Total	25·7	20·1	26·0	22·3	18·8	22·4	22·6	4·8	5

(*c*) Percentage sterility or blindness (excluding animal origin)

(1) Samples from panicles which emerged on 18–19 June									
Main	3·9	0·9	8·3	7·3	9·4	5·3	5·9	5·4	5
Bosom	5·5	5·3	14·9	9·9	11·5	8·2	9·2	Nil	—
Total	4·8	3·3	12·2	8·5	10·6	6·8	7·7	5·6	5
(2) Samples from panicles which emerged on 24–25 June									
Main	5·5	2·0	11·6	12·1	15·2	3·3	8·3	7·0	1
Bosom	8·8	5·1	17·0	13·9	18·1	7·7	11·8	Nil	—
Total	7·2	3·8	14·4	13·1	17·0	5·6	10·2	7·0	1
(3) Samples from all panicles which emerged between 7–27 June									
Main	5·1	2·0	10·4	10·3	16·1	4·1	8·1	5·3	1
Bosom	8·3	4·1	13·0	14·5	11·6	7·7	9·9	5·1	1
Total	6·7	3·1	11·9	12·6	13·3	6·0	9·0	4·4	1

Significant variations in sterility are demonstrated by the data given in Table 16(*c*), main grains showing a significant extreme difference of 14·1 %. Plot variations are very high, the standard error per plot varying from 42·2 to 78·5 %. Both the 18–19 and 24–25 June samples give results of the same order as the samples from the whole yields, and differences from the means are of the same order and significance for each variety in each series. The

as Victory, which seems likely from field observations, then segregation may be markedly transgressive.

Cunliffe (1928) showed that normal nitrogenous dressings applied about sowing time did not affect extent of shoot infestation; an excess of nitrogen significantly reduced extent of grain infestation during a season when the extent of infestation was below normal. As this might have been due to the

later growth stages having access to ample nitrogen supplies an estimation of the effect of applications of nitrogenous dressings at different growth periods on the extent of frit infestation was made with Spet × Star (Sandford) material, the square being completed with a Summer × Star selection, undressed. The square was dressed with normal quantities of superphosphates and potash and was sown on 30 March. Nitrate at the rate of 1 cwt./acre was applied in solution to four sets of plots, namely, when the coleoptile showed, when the first tiller appeared, when maximum tillering occurred and lastly when shooting first occurred, one set of Sandford being used as a control. The plants appeared above ground on 11 April, commenced to tiller on 6 May, reached the maximum tillering period about 5 June and commenced to shoot very evenly on 15 June, panicles being evenly and fully exposed on 26 June. The outer rows of the plots were discarded and record made of the numbers of plants at harvest, the number of good panicles per plant at harvest, the yield per plant and the attack on and sterility of main and bosom grains as determined by examination of the best panicles. The results are set out in Table 17.

The inference is that, although the nitrogenous dressing increased the number of good panicles per plant at harvest when applied early and the yield per plant at harvest in every case, it had no effect on either extent of grain infestation or sterility whenever applied.

Recovery capacity of Sandford compared with that of a good tillering variety (Gris de Houdan)

Cunliffe (1925) studied the extent of the recovery capacity of oats after injury similar to that caused by *O. frit*, the larval attack being simulated by killing the growing points of various stem groups of Scotch Potato and Sir Douglas Haig, these being prolific and poor tillerers respectively. Unlike Potato, Haig showed marked powers of recovery after the loss of the primary shoot; Potato yielded only 45% of its control number of spikelets per plant, whereas Haig recovered to the extent of 76%; yield of grain was affected similarly, recovery for Potato and Haig being 42 and 81% respectively. The recovery capacity of the Spet × Star selection, Sandford, was compared with that of a good tillering variety, Gris de Houdan, in a similar way during 1937. The shoot populations were artificially controlled by constantly cutting back (*a*) the primary shoot and (*b*) the primary shoot and first tiller of the plant, otherwise the crop being allowed to go to harvest

Table 17. *Effect on infestation and sterility of nitrogenous dressings at various leaf stages*

		Spet × Star (Sandford)					
Time of dressing	Nil	Appearance	1st tiller	Max. tillering	Shooting	Summer × Star Nil	Sig. diff.
No. of plants per plot at harvest	83·5	82·7	85·0	85·5	82·5	75·2	—
No. of good panicles per plant at harvest	0·81	1·25	1·07	0·84	0·92	0·62	0·15
Yield per plant (g.)	0·90	1·44	1·43	0·99	1·01	1·11	0·07
% fritted main grains	29·9	25·5	29·2	30·4	27·9	26·8	Nil
% fritted bosom grains	9·1	7·7	7·9	9·5	9·3	9·6	Nil
% sterile main grains	8·0	9·1	8·5	8·5	7·7	9·9	Nil
% sterile bosom grains	9·7	9·3	9·2	10·3	9·2	12·2	Nil

Table 18. *Recovery capacity after simulating loss of shoot by infestation*

	Stand in May	Plant survival at harvest	Panicles per plant	Spikelets per panicle	Yield of viable seed (g.)	1000 grain weight	Husk (%)
Houdan, control	79	79	4·4	28	365	31·9	20·2
Houdan, primary cut	80	79	4·5	23	288	32·3	20·0
Houdan, primary and 1st tiller cut	76	65	5·8	15	139	31·0	19·5
Sandford, control	83	83	3·0	26	337	38·9	25·4
Sandford, primary cut	81	68	4·2	17	194	38·4	26·1
Sandford, primary and 1st tiller cut	78	58	4·4	14	116	37·3	25·9
Value of *Z*	0·384	1·260	1·106	1·673	1·760	2·182	2·372
	Value of *Z* for 1% distribution, throughout, 0·7058						
Sig. diff.	—	8	0·8	4	52	1·1	0·8

for measurement of (1) plant survival, (2) panicle production, (3) spikelet production and (4) yield. A Latin square, in which seed spacing was 2 × 6 in., of six replications of each treatment was sown on 3 March to avoid natural infestation of the primary shoot, the 6th-leaf stage being reached on 10 May; even so, Gris de Houdan showed an average attack of 5 % on its primary shoots. On the treated plots cutting was started on 5 May, tillering having commenced 26 April; all tillers therefore were subject to attack throughout their susceptible stage. The essential data are set out in Table 18. Sandford was less able to withstand attack after this severe treatment, plant survival at harvest being 16 and 26 % less for the two treatments respectively, whereas Houdan remained unchanged after the first treatment and only lost 14 % after the second.

conditions simulating 100 % infestation of primary shoot, its smaller tillering capacity affecting it adversely. These data have been compared with a similar set obtained under conditions of natural infestation and recovery to determine their reliability (see Table 19). A Latin square containing these two varieties and sown late, namely, 19 April, to ensure heavy natural infestation contained six replications of each type, each plot carrying 90 seeds spaced 2 × 6 in. as before.

Sandford and Houdan lost 44 and 63 % of their primary shoots respectively. Calculating the yields of these two varieties from the data provided by the previous experiment, assuming losses of the above order in each case but ignoring complete loss of plant which is abnormal, Houdan with 100 % loss of primary shoot lost 77 g. and with a 63 % loss would

TABLE 19. *Recovery capacity under natural conditions after natural infestation*

	Stand in May	Plant survival at harvest*	Loss of primary shoot (%)	Panicles per plant	Spikelets per panicle	Yield of viable seed (g.)	Yield of seed held by no. 2 sieve	1000 grain weight	Husk (%)
Summer × Star (*a*)	80	83	47·9	2·3	33	270	234	38·6	26·1
Summer × Star (*b*)	80	82	37·4	3·0	22	149	142	42·8	24·3
Summer × Star (*c*)	85	86	36·2	2·8	37	326	205	34·8	28·4
Sandford	86	89	43·7	3·6	19	264	224	39·8	27·1
Victory	84	86	62·3	2·4	25	204	171	36·6	26·7
Gris de Houdan	83	84	63·1	4·3	22	264	69	32·6	20·2
Value of *Z*	—	0·566	1·235	2·061	1·934	1·452	1·700	2·198	2·229
	Value of *Z* for 1 % distribution, 0·7058 throughout								
Sig. diff.	—	—	9·7	0·3	2·9	41	32	1·2	0·9

* The discrepancy is due to breakage; complete loss of plant, such as occurred in the previous experiment with Sandford, is abnormal under such light attack.

The limit of panicle production was not reached, the total production of panicles per plot showing very little variation (Houdan, 347, 356 and 377 and Sandford, 249, 286 and 255 for controls and treatments, respectively). After loss of the primary shoot only, Sandford produced 91 % of the control number of spikelets per plant against 84 % for Houdan. The effect of the treatment on yield per plant was somewhat similar in each case; after the first treatment, yield per plant dropped by 22 and 29 % for Houdan and Sandford respectively, both resembling Haig, and after the double cut by more than 50 % in each case. These losses occurred under wide spatial conditions, which would tend to encourage recovery much more than would be the case under normal field spacing. Thousand-grain weight and percentage of husk were practically unaffected, the third and possibly subsequent shoots being capable therefore of producing some grain of quality within the more limited growth period. Thus Sandford did not exhibit the recovery capacity of Houdan under

have lost approximately 48 g., leaving a yield figure of 317 g. per plot; Sandford, with a 100 % loss of primary shoot, lost 100 g. proportionally and with a 44 % loss would have lost approximately 44 g., leaving a yield figure of 293 g. per plot, which is very close to that of Houdan as was the case under conditions of natural infestation, where yields of viable seed were equal. Thus this method of estimating comparative recovery capacity has a considerable degree of accuracy.

The Summer × Star selections, *a*, *b* and *c*, show the variability of closely related selections, although under these unknown conditions it is impossible to say how far yield is influenced by recovery capacity as opposed to other agricultural qualities.

The great importance of the primary shoot is apparent, and obviously any degree of resistance to attack possessed by a variety is of value because it tends to safeguard this shoot, although some varieties are able to recover to a considerable degree from the loss of the primary shoot, yielding good-quality grain.

Varietal differences in extent of resistance to infestation exhibited by maize

There are occasional references in the European literature to maize infestation by *O. frit*; this source of damage to maize has been brought into prominence by efforts made to improve maize varieties for utilization in this country. The question arose as to whether injury was caused by the fly and, if so, whether varietal differences existed.

Observation and breeding proved that frit fly was alone responsible. Little was known of the extent of damage or of the type of damage; consequently mainly for experience, sowings were made from five types of maize in rows of ten seeds, with three replications, on six dates between 10 May and 5 June at intervals of 5 days, all randomized. It was possible to arrange one small Latin square and this was set out on 5 June, on which date main sowings are apparently normally made. Later sowings failed completely, even when protected between two rows of Victory.

A certain amount of difficulty was experienced in deciding what was to be recorded as an attacked shoot. The rapidity of growth of the plant sometimes appeared to carry the larva out of reach of the growing point, but the massiveness of the plant enabled the larva to obtain sufficient nourishment locally, which led to blade damage only. It was expected that this would have been immaterial to spikelet production, but it appears that even plants so damaged lose their commercial value. The records show that even apparently healthy shoots 12 in. high may contain larvae which have destroyed the growing

Table 20. *Differential resistance of maize varieties to infestation by the frit fly*

(*a*) Random row experiment: loss of primary shoot (%)

	Differences from means of blocks					
Date of sowing	Gemcross C13	Sencross 39	Golden Cross Bantam P39×P51	Dorinny ×C13	First of All	Mean of block
			(1) Excluding blade damage			
10 May	+15·4	+ 1·5	−26·1	−20·5	+29·2	44·8
15 May	+16·5	−28·2	−13·9	+13·8	+11·8	51·5
20 May	+12·3	−32·0	− 9·7	+ 5·0	+24·3	58·7
25 May	+19·9	−20·1	−17·4	− 7·4	+24·9	63·1
30 May	+ 5·6	−20·0	+ 5·0	+ 1·6	+ 6·0	84·7
5 June	+ 7·2	− 1·4	+ 4·9	−11·1	+ 0·2	48·1
Mean	+12·8	−16·7	− 9·5	− 3·1	+16·1	—
c/f Sencross 39 *D/E*	4·72	—	0·83	2·03	4·51	—
	The value 2·57 representing 100 : 1 chance					
			(2) Including blade damage			
10 May	+14	−10	−21	+ 6	+12	75
15 May	+13	−23	+ 5	+ 3	+ 4	71
20 May	+11	−22	0	+ 7	+ 5	82
25 May	+ 3	− 7	−10	+ 9	+ 6	86
30 May	+ 2	− 7	+ 7	− 4	0	93
5 June	0	− 1	+ 5	−13	+ 5	62
Mean	+ 7	−12	− 2	+ 1	+ 5	—

(*b*) Latin square experiment, including Victory oats

Individual strains: loss of primary shoot (%)

Gemcross C13	Sencross 39	Golden Cross Bantam	Dorinny ×C13	Victory	Sig. diff.
		(1) Excluding blade damage			
18·7	9·6	15·7	19·5	70·6	8·5
		(2) Including blade damage			
45·8	33·4	40 4	46·7	70·6	12·1

point; conversely, stunted shoots, which on visual examination alone would be recorded as damaged, may be found on detailed examination to be free from larvae and without any internal evidence of boring. It is therefore essential to open and examine every stem carefully for evidence of larval operation before recording. The observations set out in Table 20(*a*) were made on types of interest in current breeding work, the table being self-explanatory. They show that Sencross 39 is more resistant than Gemcross C 13 and First of All, but is not differentiated from Golden Cross Bantam and Dorinny × C 13. These figures must be considered with caution, because considering each sowing as a single randomized block experiment, those of 10 and 30 May and 5 June are not significant, *Z* being of too low a value; this, of course, may easily occur even with large mean differences. Those of 20 and 25 May are only significant at the 5 % point of distribution, while that of 15 May is significant at the 1 % point of distribution.

The Latin square of 300 seeds of each variety in five replications, sown on 5 June, included Victory to give some idea of fly prevalence. The experiment is clearly significant (see Table 20(*b*)). The attack on Victory was low for the time of planting, but the plot was protected by tall oats from fly to some extent; it was shown in 1935 that between 1 and 2 ft. of lateral cover greatly reduced extent of attack in small plots (Cunliffe, 1936). Victory was significantly higher than any of the maize varieties; of the latter Sencross 39 was significantly below Gemcross C 13 and Dorinny × C 13, but not differentiated from Golden Cross Bantam.

This more precise experiment confirms the result of the row experiment, in so far as Sencross 39 is shown to be more resistant than Gemcross C 13, but Dorinny × C 13 is now classed with the latter instead of the former. Golden Cross Bantam, as before, is not differentiated from Sencross 39. This result follows the line of the 15 May row experiment, which it will be remembered was the one significant at the 1 % point of distribution. Gemcross C 13 and Dorinny × C 13 being early maturing varieties, it is possible that further experiment would show a positive correlation between early maturity and extent of infestation.

The extent of blade damage which will cause commercial loss is unknown, and, as it may vary from tip damage only to complete distortion of blade, estimates of damage including blade damage are at the moment of comparatively little value. The data to the nearest whole figures obtained are included here for record but without statistical analysis except for the Latin square (see Table 20). The Latin square experiment is clearly significant, and both have the general trend of those excluding blade damage.

Repellents

In particular cases, as, for example, with late-sown plants of known oviposition history, it is frequently necessary to obtain seed for further observations; covering is unsatisfactory in every way, and an effective repellent would be very useful under experimental conditions.

The fly does not seem to be sensitive to any marked degree to common repellents, such as whizzed naphthalene, methyl salicylate, paraffin, turpentine, cinnamon and camphor. Heavy dressings (½ lb. or ½ pint per 150 plants) were applied between the plants on 11 May 1940 to plots of 150 Victory plants sown on 1 May. Cinnamon, methyl salicylate and camphor were still obvious on 27 May. Lateral effects were severe in three cases, in which the stands were reduced to 44–64 plants.

Three interspersed Victory control plots, with an average stand of 134 plants, showed a mean loss of primary shoot of 97·6 %. Cinnamon was the most effective repellent, but it only reduced the infestation by 21·3 %; smaller significant differences were brought about by methyl salicylate and paraffin, while camphor, turpentine and naphthalene were quite ineffective.

Heavy dressings of D.D.T. were more effective, although here the action may be lethal rather than repellent. During 1945, a Latin square of 60 seeds per plot was sown on 5 June, i.e. very late, to determine whether D.D.T. would protect experimental material required to go to harvest. It included the extreme varieties, Victory and Summer, each providing material for controls, and for treatment with kaolin base alone, and 5 % dust; in addition, Victory was treated with 30 % dust and 0·1 % spray made from the Bristol formula; this spray burnt the foliage severely. The material was treated five times, as determined by weather conditions. Heavy dressings afford substantial, but not complete, protection as shown by the following data, which indicate the percentage losses of primary shoots, for individual treatments:

	Control	Base	D.D.T. 5 %	D.D.T. 30 %	D.D.T. 0·1 % spray
Victory	99·1	98·8	74·1	32·8	33·3
Summer	77·8	71·8	30·0	—	—

Significant difference 7·3.

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A survey of potato aphides in north-west Derbyshire, 1945

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(With 1 Text-figure)

Counts of aphides infesting potato crops in north-west Derbyshire at altitudes between 340 and 1360 ft. were made during the summer of 1945. *Myzus persicae* was abundant on almost all crops, and peak figures on some reached over 1500 per 100 lower leaves. In many fields two peaks were noted, one towards the end of July and the second in mid-September, and in some fields there were still heavy infestations when the haulms died. The number of aphides varied considerably from field to field; sheltered fields were the least infested. In this area in 1945 there was no evidence to support the contention that the altitude and aspect of the field have any influence on the intensity of infestation. Tuber samples from six fields showed that there was a considerable spread of leaf roll.

Introduction

Under the Ministry of Agriculture and Fisheries scheme for the inspection of growing crops of potatoes (1945), areas in northern England with an altitude over 400 ft. are eligible for the certification of high-grade seed potatoes. Existing knowledge suggested that districts of this type would be unfavourable for the flight and rapid multiplication of *Myzus persicae* Sulz., and hence would be districts where virus spreads slowly. North-west Derbyshire is included in the area covered by the scheme, and the present survey was therefore made to obtain some knowledge of the distribution of the potato aphides. Previous surveys of a similar nature are listed by Jacob (1944).

Data relating to the spread of virus diseases in crops upon which aphis counts have been made are given in an addendum, though no attempt is made in this paper to prove or disprove the suitability of the area for the growth of certified seed potatoes, nor is it suggested that the conditions in 1945 were similar to those which normally prevail.

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Description of the area surveyed

With the exception of the lower part of the Derwent valley system the whole area lies above 400 ft. (see Fig. 1). It forms the southern spur of the Pennines, running N.N.W.—S.S.E., with the centre area of carboniferous limestone and limestone shales, flanked by millstone grit on the west and east. The Derwent valley separates the millstone grit ridge to the east from the limestone uplands. The basic rocks are usually near the surface, so that, except in the river valleys, the soil is derived from the underlying rocks.

The average annual rainfall is over 30 in., rising to 50 in. in the north-west. There is a temperature gradient from west to east, the western part of the area being 2–5° F. lower than the eastern throughout the year. Westerly winds prevail.

It is a non-industrial area, but is bounded by industrial districts in the north-west (Manchester), north-east (Sheffield) and east (Chesterfield). Numerous villages and a few small towns are scattered over the area (apart from the uncultivated High Peak in the north it is impossible to find a field which is more than two miles from a village or small settlement). Prior to the recent war most of the land was devoted to grazing and dairy farming. During the war much land was broken up for arable crops, and the potato acreage increased from about 2000 to about 11,000.

Of potato seed used, 25–40% is annually imported from Scotland or Ireland. One or two farmers have been in the habit of selling seed (once or twice grown from a good Scotch stock), and this has a good reputation amongst their customers.

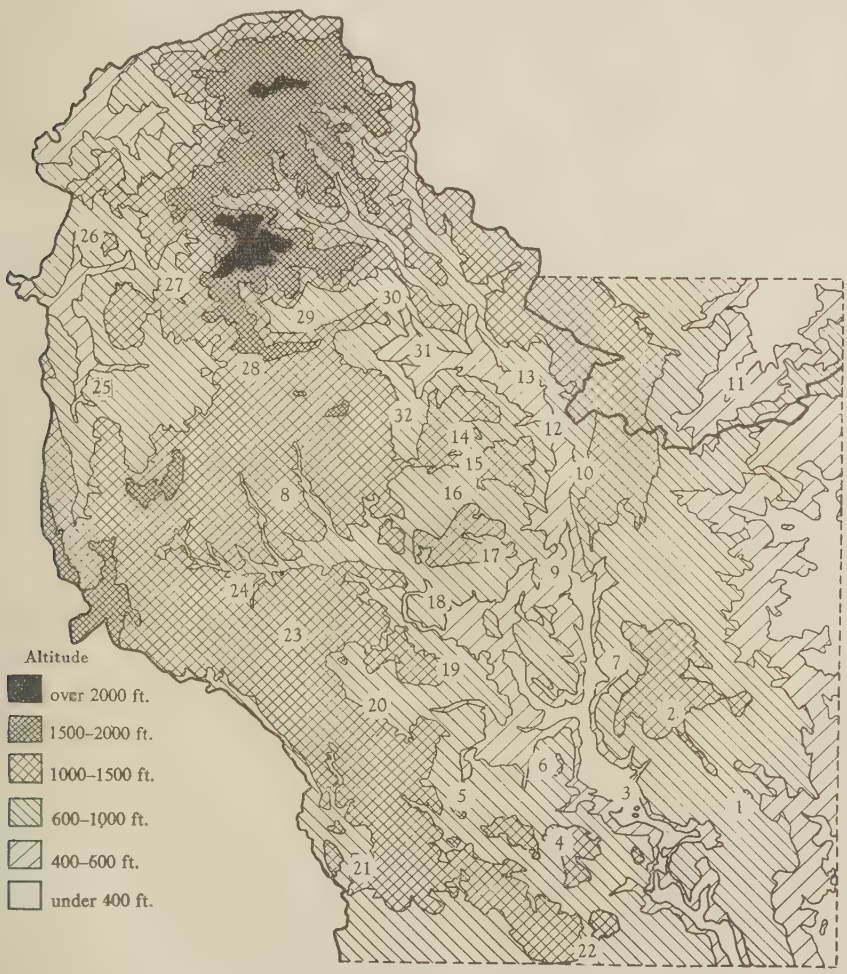

Fig. 1. Map of the area surveyed showing the sites of the fields studied.

Methods used and details of the survey

The aphides considered were *Myzus persicae* Sulz., *Aulacorthum solani* Kalt. (=*Myzus pseudosolani* Theo.), *Macrosiphum solanifolii* Ash. (=*M. gei* Koch partim), and *Aphis* (*Doralis*) *rhamni* B.d.F.

Thirty-two fields of potatoes were visited at intervals of 1–3 weeks from the beginning of June to the end of September (Fig. 1). They were scattered over the whole area and chosen to include as many different conditions as possible. Eleven fields

were over 1000 ft., eighteen between 500 and 1000 ft., and three were below 500 ft. Following a very limited survey of field Brassicae during the later part of the previous winter, when very few overwintering *Myzus persicae* were found, and taking into consideration the topography of the area, it was considered likely that the number of aphides to be found on the potatoes during the summer might be low, and because *M. persicae* occurs more frequently on lower leaves than on middle or upper the number of aphides per 100 lower leaves picked at random from 100 plants in a traverse of the crop was adopted as the standard for the counts. During the season, however, *M. persicae* increased to such an extent that it was possible to count fifty leaves only in many fields during the peak period of infestation.

fields at high altitudes. The total catches of aphides from these traps are described elsewhere (Broadbent, 1946), but the catches of potato aphides are tabulated in Table 1. *Aphis rhamni* could not be distinguished with certainty from other *Aphis* spp. after removal from the trap.

Alatae and apterae of each species were recorded separately; the percentage of leaves infested with aphides was noted, as well as coccinellid, braconid and syrphid parasites and predators. Records of the force and direction of the wind, the air temperature, and the relative humidity (by means of a whirling hygrometer) were taken at the time of each count.

In the limited space available it is not possible to give the detailed data for each field, but some comparison between the different sites may be obtained

TABLE 1. *Potato aphides trapped in potato fields*, 1945

Brackets indicate the lengths of time during which the traps operated. Figures or dashes show the weeks during which the traps were cleared of aphides

	May				June				July				Aug.				Sept.				Oct.				Nov.				
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	Total
Myzus persicae Sulz.																													
Field 2	[.	.	.	—	—	—	—	1	—	2	24	14	4	36	.	8	4	2	2	—	2	2	.	4]	.	.	.	.	105
3	[.	.	.	—	—	—	—	—	2	32	36	26	15	40	.	10	21	31	5	7	3	—	.	1	.	—]	.	.	229
11	[.	.	.	—	—	—	2	—	—	—	32	4	8	12	2	3	1	2	1	1	7	8	.	—	.	2]	.	.	85
12	[.	.	.	—	—	—	—	—	—	—	—	2	2	2	2	1	1	1	—	—	6	4	.	—	.	1]	.	.	22
15	[.	.	.	—	—	—	1	—	—	4	32	2	6	14	5	—	1	4	7	2	—	3	.	—	.	—]	.	.	81
23	[.	.	.	—	—	—	—	—	1	4	4	8	20	19	5	2	4	5	1	2	1	—	.	—	.	—]	.	.	76
Totals	.	.	.	—	—	—	3	1	3	42	128	56	55	123	14	24	32	45	16	12	19	17	.	5	.	3	.	.	598
Macrosiphum solanifolii Ash.																													
Field 2	[.	.	.	—	—	—	—	—	—	—	4	—	2	4	.	—	—	—	—	—	—	—	.	—]	.	.	.	.	10
3	[.	.	.	—	—	—	6	—	—	—	—	—	—	4	.	—	1	—	—	1	—	—	.	—	.	—]	.	.	12
11	[.	.	.	—	—	2	4	—	2	2	—	2	2	—	—	—	3	—	1	—	—	—	.	—	.	—]	.	.	18
12	[.	.	.	—	—	—	—	—	—	2	8	—	—	—	—	—	—	—	—	—	—	—	.	—	.	—]	.	.	10
15	[.	.	.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	.	—	.	—]	.	.	—
23	[.	.	.	—	—	—	—	—	—	2	2	—	—	—	—	—	—	—	—	—	—	—	.	—	.	—]	.	.	4
Totals	.	.	.	—	—	2	10	—	2	6	14	2	4	8	—	—	4	—	1	1	—	—	.	—	.	—	.	.	54

On these occasions the leaves were taken in batches of twenty-five, and if there was a significant difference between the total populations of aphides on two batches of twenty-five leaves the counts were extended to 100 leaves. All numbers recorded in the tables are on a common basis of aphides per 100 lower leaves.

In order to have some comparison with previous similar surveys 100 lower and 100 middle leaves were counted on the same date in seven fields about the middle of July, at, or just before, the peak infestation. The average count of *M. persicae* per 100 lower leaves was 888, with 93% of the leaves infested; the average count on the middle leaves was 316, with 56% of the leaves infested.

Six 'stove-pipe' adhesive traps were erected, three in potato fields at low altitudes, three in potato

from Table 2 which gives the highest count recorded for each of the four species and the date of the count. These counts may, or may not, have been at the peak of the infestation, since in some fields only four or five counts were made during the season, at intervals of 2–3 weeks. In the six fields with traps, however, counts were made nearly every week and the details of these counts are given in Table 3.

WEATHER CONDITIONS DURING THE EARLY SUMMER MIGRATION

The arbitrary standards of conditions most suitable for aphis migration were discussed by Thomas & Vevai (1940), who suggested the following:

(*a*) a temperature above 70° F.,
(*b*) a relative humidity below 80%,
(*c*) a wind velocity below 5 m.p.h.,

(*d*) a difference in maximum and minimum temperatures of more than 12° F.

Taking the average conditions over the area surveyed (based on the daily weather records of Ashbourne, Buxton and Sheffield) and applying the above standards, the number of days suitable for flight in 1945 would be two in May, none in June and two in July.

The spring of 1945 in Derbyshire was exceptionally mild, with dry conditions in March and the temperature in April above normal. These conditions would encourage any aphides which had overwintered to build up large populations, and it may be presumed that a large reservoir of *Myzus persicae* was available for infesting the potatoes. May and June were unsettled and wet, though there were two showers only during the 8 days 13–20 June, the week during which many of the crops first became infested. The average weather conditions during this week were:

(*a*) maximum temperature 66° F. (2 days above 70° F.),

(*b*) minimum relative humidity (computed) 64 %,

(*c*) wind from the west from 13–18 June, from the east on the 19th and 20th; velocity above 10 m.p.h. except on 20 June,

(*d*) a difference of 18° F. between maximum and minimum temperatures.

Apart from the average maximum temperature and wind velocity the general conditions during this week were suitable for aphis flight, and it can be assumed that during this time periods of calm occurred which would encourage aphides to take to the wing.

The average conditions during the remainder of the month, during which time aphides continued to infest potatoes, were:

(*a*) maximum temperature 64° F. (3 days above 70° F.),

(*b*) minimum relative humidity (computed) 70 %,

(*c*) wind mainly from the west, but many periods with velocities below 5 m.p.h.,

(*d*) a difference of 14° F. between maximum and minimum temperatures.

TABLE 2. *Number of aphides per* 100 *lower leaves at the peaks (approximately) of the different infestations*

Field											
No.	Aspect	Altitude (ft.)	Maximum % leaves infested	*M. persicae*		*A. solani*		*M. solanifolii*		*A. rhamni*	
1	S.W.	950	100	26 July	876	26 July	12	26 July	256	13 Aug.	82
2	L.	1000	90	21 July	620	1 Aug.	7	1 Aug.	70	27 Aug.	5
3	N.E.	340	100	21 July	1312	26 July	18	21 July	680	1 Aug.	368
4	L.	1000	100	26 July	998	—		26 July	48	16 Aug.	2
5	N.E.	950	92	28 July	526	—		28 July	24	15 Aug.	8
6	W.N.W.	950	98	1 Aug.	1228	12 July	23	1 Aug.	56	23 Aug.	10
7	S.W.	600	98	28 July	788	6 Sept.	12	28 July	64	16 Aug.	1
8	L.	1000	100	24 Aug.	1330	12 Sept.	2	20 July	78	24 Aug.	2
9	S.E.	500	100	26 July	2216	26 July	36	11 Aug.	302	27 Aug.	64
10	N.W.	950	79	26 July	297	4 July	12	26 July	54	26 July	5
11	L.	400	54	10 Aug.	98	21 June	9	24 Aug.	34	24 Aug.	2
12	N.E.	460	80	21 July	164	21 July	6	21 July	112	1 Aug.	8
13	E.	750	90	2 Aug.	510	31 Aug.	4	20 July	66	—	
14	L.	1000	100	25 July	1672	25 July	2	25 July	162	7 Sept.	4
15	L.	1280	100	21 July	1116	28 July	2	21 July	62	1 Sept.	26
16	L.	900	100	25 July	1926	—		25 July	126	1 Sept.	52
17	N.E.	1050	100	28 July	628	28 July	10	28 July	18	11 Aug.	22
18	E.	800	100	13 Sept.	1126	1 Aug.	12	1 Aug.	48	23 Aug.	7
19	E.	1000	100	11 Aug.	1528	20 July	4	20 July	120	23 Aug.	16
20	N.E.	950	99	18 Sept.	824	2 Aug.	1	19 July	183	31 Aug.	14
21	E.N.E.	1030	100	19 July	1032	—		19 July	193	15 July	6
22	N.E.	930	90	26 July	688	16 Aug.	2	26 July	17	6 Sept.	34
23	W.	1260	98	25 July	1274	—		19 July	71	—	
24	S.	1000	92	19 July	948	4 July	1	19 July	80	—	
25	S.W.	800	84	20 July	573	20 July	2	20 July	121	10 Aug.	8
26	W.	800	90	25 July	508	—		25 July	34	—	
27	W.	950	96	12 Sept.	1870	25 July	2	25 July	8	12 Sept.	2
28	L.	1360	100	24 Aug.	1068	7 Sept.	1	20 July	65	2 Aug.	2
29	N.	900	89	25 July	325	25 July	5	25 July	26	7 Sept.	16
30	N.E.	800	94	2 Aug.	568	20 July	3	20 July	57	—	
31	L.	540	93	2 Aug.	588	20 July	4	20 July	199	12 Sept.	2
32	E.	940	86	19 Sept.	544	25 July	1	25 July	9	—	

On 6 of the 11 days heavy rain was recorded; nevertheless, conditions were occasionally ideal for flight.

There followed a rainless period from 2 to 8 July, when the maximum temperature averaged 68° F. (with 2 days above 70° F.), the minimum humidity 65 % and westerly winds of about 10 m.p.h. velocity were fairly constant.

The usual climatic conditions of the district are considered to be unfavourable for aphis flight, e.g. of the 41 days spent by the writer in the area during the summer, when frequent estimates of the wind speed were made, only 5 days of calm (velocity below 5 m.p.h.) were experienced, while winds of over 15 m.p.h. occurred on 27 days. There were only 5 days in June when the temperature reached or exceeded 70° F. and 11 in July. Nevertheless, conditions must have been favourable on numerous occasions during the periods described above. Furthermore, the following evidence shows that aphides do fly under seemingly very adverse conditions.

On 7 July 1945 four cylindrical traps 12½ in. in diameter and 3 in. in depth were erected on posts in the middle of a 17-acre field of potatoes at the Midland Agricultural College, and were coated with a grease-banding substance. The traps were 18, 36, 48 and 48 in. from the ground. The total catches for the day showed no significant differences between the traps at the different heights. The experiment was repeated on 10 July under very different weather conditions and with the four traps one above the other on one post, at heights of 30, 48, 60 and 72 in. above the ground. On this occasion twice as many

TABLE 3. *Number of aphides per 100 lower leaves in the six fields with traps. Counts throughout the growing season*

Field and altitude	Date 1945	Leaves infested (%)	*M. persicae*	*A. solani*	*M. solanifolii*	*A. rhamni*
2	13. vi	—	—	—	—	—
1000 ft.	21. vi	2	3	—	—	—
	26. vi	6	10	—	—	—
	3. vii	5	3	—	1	—
	12. vii	39	74	1	25	—
	21. vii	84	620	—	52	—
	26. vii	90	556	2	58	—
	1. viii	85	363	7	70	—
	13. viii	86	312	—	34	2
	27. viii	28	34	—	8	5
	6. ix	16	28	—	—	—
	19. ix	16	14	—	8	—
	27. ix	—	—	—	—	—
3	13. vi	—	—	—	-	—
340 ft.	21. vi	9	25	1	—	—
	26. vi	23	51	1	—	—
	4. vii	47	123	2	8	1
	13. vii	88	392	14	124	11
	21. vii	100	1312	12	680	112
	26. vii	98	1272	18	370	352
	1. viii	96	644	16	188	368
	13. viii	100	662	4	196	352
	27. viii	98	348	4	136	196
	6. ix	86	550	—	62	112
	19. ix	94	792	—	168	18
	27. ix	—		—	—	—
11	4. vi	—	—	—	—	—
400 ft.	13. vi	4	6	—	-	—
	21. vi	10	12	9	2	—
	27. vi	22	42	—	17	—
	4. vii	31	41	4	20	—
	12. vii	34	41	8	24	—
	21. vii	22	16	2	4	—
	26. vii	46	38	2	28	—
	10. viii	54	98	—	22	—
	24. viii	42	84	—	34	2
12	20. vi	5	4	1	—	—
460 ft.	26. vi	17	36	—	3	—
	4. vii	27	66	—	1	—
	12. vii	49	132	6	31	7
	21. vii	66	164	6	112	—
	28. vii	72	136	2	32	—
	1. viii	54	104	—	34	8
	10. viii	53	65	1	31	—
	16. viii	66	141	2	89	—
	24. viii	80	148	4	84	—
	7. ix	62	88	2	56	8
	18. ix	64	154	—	106	—
	27. ix	—	—	—	—	—
15	12. vi	—	—	—	—	—
1280 ft.	26. vi	19	38	—	—	—
	3. vii	15	18	—	—	—
	11. vii	82	354	—	7	—
	21. vii	100	1116	—	62	—
	28. vii	96	714	2	28	—
	1. viii	92	748	—	28	—
	11. viii	90	598	—	12	—
	16. viii	89	621	—	5	1
	23. viii	88	676	—	22	—
	1. ix	86	602	—	2	26
	7. ix	74	576	—	14	2
	13. ix	81	435	—	2	5
	19. ix	80	580	—	8	—
	28. ix	74	508	—	4	—
	3. x	A few aphides on the few leaves still left				
23	11. vii	60	218	—	12	—
1260 ft.	19. vii	91	463	—	71	—
	25. vii	98	1274	—	62	—
	2. viii	97	615	—	52	—
	15. viii	90	414	—	24	—
	24. viii	84	314	—	4	—
	31. viii	55	161	—	2	—
	12. ix	22	59	—	—	—
	25. ix	—	—	—	—	—

aphides were caught on the upper two traps as on the lower two. The traps were visited at 2-hourly intervals throughout the day, all the aphides being removed at each visit. The catches and the weather conditions are summarized in Table 4, which shows that fairly large numbers of aphides were airborne above a potato crop under the following conditions:

(*a*) at temperatures below 63° F.,
(*b*) with the relative humidity as high as 95%,
(*c*) with wind velocities up to 20 m.p.h.,
(*d*) during continuous light rain.

Since Maldwyn Davies (1936) showed conclusively that *M. persicae* would not undertake voluntary flight at wind speeds of more than 4 m.p.h. under laboratory conditions and could not be blown off surfaces to which they were clinging, even by hurricanes of 70 m.p.h., it follows that the conditions in the crop, from which the aphides presumably took off, must have been very different from those which were measured above the crop. This is also borne out by the fact that during the third week of July more *M. persicae* were taken on the traps (see Table 1) than during any other week, despite the high winds which prevailed at that time.

OVERWINTERING OF *MYZUS PERSICAE*

It was obvious from the large numbers of *M. persicae* present during the summer that a very inadequate picture of the overwintering of this species had been obtained by the cursory examination of a few field Brassicae during the early months of the year. During February and March 1946, while working at the Rothamsted Experimental Station, the writer had the opportunity of visiting on a few occasions the area surveyed during the previous year, and of assessing to some extent the possibilities of overwintering.

Brassicae, especially cabbages, are grown by most Derbyshire farmers for cattle food, but are usually cut and consumed by the end of January at the latest. A few were found here and there in the middle of February 1946, but no aphides could be found on them. Some Brassica crops in gardens in various parts of the district were examined, with the results as shown below.

Those found were all under the outer leaves near the ground, for as Ludwig (1945) pointed out, during exposure to low temperatures a moist environment is beneficial because the latent heat of water prevents a very rapid drop in temperature.

M. persicae was also found on tulips and other plants under glass, though not frequently. There are few peach trees in the area, and they were visited during the autumn of 1945 and the spring of 1946. Oviparae and eggs were found in the autumn, but since all the growers make it a routine practice to spray or wash their trees during the winter, there is little chance of these trees providing the source of the summer infestation. In one large glasshouse small ornamental peaches in tubs were found to be infested with oviparae and eggs in the autumn and with fundatrices in the spring, but this type of tree is rare in the locality.

It seems likely that the source of infestation is in the numerous gardens and glasshouses of the villages, where, except in the most severe winters, *M. persicae* can survive in small numbers. If only a few survived the conditions of December 1944, when there was ground frost on 17 days, and of January 1945, when there was ground frost on 22 days and snow lying on 20 days, the exceptionally mild spring would have enabled them to multiply and spread over the area before the potatoes were planted.

DISCUSSION

The counts show that the summer of 1945 was very favourable for *M. persicae* on potatoes in Derbyshire. In every field studied this was the predominant species and the other species reached large numbers in a few fields only.

		M. persicae per 100 leaves	% of leaves infested
13. ii. 46	Cabbages (Jan. King)	44	32
	Savoys	28	16
	Broccoli	0	—
	Sprouts	0	—
	Kale	0	—
14. ii. 46	Savoys	0	—
	Broccoli	0	—
	Sprouts	0	—
	Kale	0	—
25. ii. 46	Savoys	36	16
	Spring cabbages	0	—
26. ii. 46	Savoys	170	30
12. iii. 46	Cabbages (Jan. King)	24	12
	Savoys	28	12
	Savoys	30	10
13. iii. 46	Savoys	60	12
	Spring cabbages	0	—

Alatae of *M. persicae* began to infest the potatoes during the third week of June. In most crops, whatever the time of planting, the peak of the infestation occurred about the third or fourth week of July. Even in field 23, where the potatoes did not begin to appear above ground until the first week of July, 98% of the lower leaves were infested and 1274 *M. persicae* per 100 lower leaves were counted on 25 July.

The trap records (Table 1) indicate two peaks in the number of *M. persicae* on the wing, during the third week of July and the second week of August. At both these periods the numbers counted on the plants also fell, and it is likely that such reductions were due to a proportion of the population leaving the potatoes.

reproductive activity before the autumn dispersal from the potatoes, just as the July peak foreshadowed a summer dispersal of alatae.

In most cases the infestation, though falling somewhat after the July-August dispersals, remained relatively high until late in the season, in some fields until only a few leaves remained alive. This would seem to indicate a lack of activity in predators and parasites, yet aphides parasitized by *Aphidius* spp. were numerous, 30–100 per 100 leaves being common, while coccinellids and syrphids usually occurred at a rate of 2–10 per 100 leaves. There was no evidence of a significantly greater number of parasites or predators on fields with a lesser number of aphides, or of fewer enemies among the higher infestations. Fungi of the genus *Entomphthora* were

TABLE 4. *Two days' trapping of aphides, showing that flight takes place under adverse weather conditions*

Time G.M.T. hr.		General conditions	Air Temp. ° F.	R.H. %	Wind. Beaufort scale	Totals (four traps) *M. persicae*	*A. solani*	*M. solanifolii*
7. vii. 45: Erected by	5.30	Sunny	59	89	S.W. 3	—	—	—
	7.30	Sunny	64	77	N.W. 4	5	—	5
	9.30	Sunny	68	69	N.W. 5	8	1	1
	11.30	Sunny	68	65	N.W. 5	11	—	2
	13.30	Sunny periods	70	61	W. 4	10	—	2
	15.30	Sunny periods	69	57	N.W. 4	5	—	4
	17.30	Cloudy	66	59	N.W. 3	11	1	1
	19.30	Sunny	61	73	N.W. 2	14	—	3
8. vii. 45:	5.00	Sunny	50	93	S.E. 1	18	—	—
10. vii. 45:	6.00	Sunny	57	94	W. 2	—	—	—
	8.00	Dull	63	84	W. 2	8	—	7
	10.00	Dull	63	86	W. 2	4	—	3
	12.00	Drizzle since 10.15	64	90	N.W. 2	6	—	6
	14.00	Continuous drizzle	63	95	N.W. 1	11	—	27
	18.00	Dull after very heavy rain	59	94	N.W. 4	4	—	13
11. vii. 45:	5.00	Very wet night after 18.00	56	100	N.W. 3	—	—	—

Table 2 shows that a number of fields had very late peak infestations. This was due to a second peak in late August or early September, which in these fields reached greater proportions than the July peak. This second peak, in some fields much higher than in others, was observed clearly in twelve fields; if it occurred elsewhere it was missed owing to the length of time which elapsed between visits. The trap records for field 3 indicate a marked increase in alatae during the first half of September which did not occur on the other traps. Table 3 shows that, of the fields with traps, field 3 was the only one to show a very marked second peak during this same period. It was noted that at these peak times a large number, often a majority, of the aphides present on the potatoes were nymphs of alatae, and presumably the autumn peak was due to a sudden increase in prevalent about the period of the July peak and helped to reduce the aphides at this time.

As records were taken on lower leaves, the ridging up of the crop early in the season reduced the count, often considerably, because the lower leaves after ridging were previously middle leaves. The time taken to build up the population again after this operation would indicate that most of the aphides buried by ridging perished.

It was obvious from the survey that *Myzus persicae* may be prevalent at all altitudes up to 1400 ft. at least. In the range of altitude surveyed there was no evidence in favour of the statement that fewer aphides occur at higher altitudes, or that the aspect of the field plays an important part in the time or intensity of the infestation. On only four fields did the population of *M. persicae* remain below 500 per

100 lower leaves, i.e. on fields 10 (297/100 leaves), 11 (98/100 leaves), 12 (164/100 leaves) and 29 (325/100 leaves). Fields 11 and 12 were in similar situations at the bottom of relatively steep-sided narrow valleys (running N.N.W.–S.S.E. and N.E.–S.W. respectively), by the side of rivers, and well sheltered by trees. Field 29 was on the southern open slope of a deep valley running west to east. Field 11 was in the midst of a residential suburb of Sheffield, and a large population of *M. persicae* was expected here, yet this remained the least infested of all the sites, despite the fact that thirty-two alatae of this species were recorded on the trap during the third week of July, a number comparable with those recorded on traps in fields 2, 3 and 15 (Table 1). (More *M. persicae* were trapped in field 11 during the season than in fields 15 and 23.) During this same week the counts of *M. persicae* on the potatoes (per 100 lower leaves) were: field 11, 16; field 2, 620; field 3, 1312; field 15, 1116. At this time large numbers of this insect were noted on young cabbages and on turnips to the east of the potatoes in field 11, and presumably it was from these crops that the trapped insects came. Brassica crops were present in many other fields, but this did not prevent *M. persicae* from establishing high populations on the potatoes. Small numbers of *M. persicae* were present on Brassicae in field 12 during the season, and the higher numbers recorded on traps 11 and 12 in October after the potatoes had been lifted no doubt came from the Brassicae. The other four traps were in fields which contained no green Brassica crops (field 3 carried a crop of swedes). The traps indicated that the least flighting took place in field 12, the most enclosed field of those studied. It may be that this species is carried by air currents over fields situated in deep valleys or areas well enclosed by trees. Hardy & Milne (1938) showed that it was wind-borne up to a height of 650 ft. above land. The fact remains that all the fields received initial infestations, and the causes of subsequent differential increases in populations were obscure.

Aulacorthum solani was recorded on twenty-six of the thirty-two fields, though in small numbers everywhere. It occurred from the second week of June to the third week of September, being most numerous during July.

Macrosiphum solanifolii occurred on all the crops, but in relatively small numbers except in the two low-lying fields, 3 and 9. In no fields were the upper parts of the plants very heavily infested as was the case at the Midland Agricultural College. They were present throughout the season from the third week of June, reaching a marked peak during the third week of July. Though by far the highest infestation (680/100 leaves) was recorded in field 3, relatively few were taken on the trap in this field.

Aphis rhamni occurred in twenty-six fields, but reached relatively high numbers in field 3 only. It was first recorded during the last week of June, and in some fields small numbers remained until the end of September. The peak of the infestation in field 3 lasted for nearly 4 weeks with little change in numbers, from 23 July to 13 August. *Rhamnus catharticus*, the winter host of *Aphis rhamni*, is not recorded as being common in Derbyshire. Most of the sites listed are in the Matlock area, which might account for the heaviest infestation being in field 3.

The writer is indebted to Mr A. Roebuck, for suggesting that the survey be undertaken and for his encouragement and helpful criticism; to Messrs L. H. Manning, L. M. Waud and W. S. English for putting him in touch with the potato growers and gardeners; to Mr and Mrs J. P. Doncaster who assisted with the counts on 19 and 20 July, and to Mr Doncaster in particular for his help in planning the survey; to Dr Glasspool, of the Meteorological Office, for placing the Derbyshire weather records at his disposal; to the farmers, garden owners and gardeners who so generously allowed him free access to their land and crops; also to his wife for her occasional assistance in the field.

ADDENDUM ON THE SPREAD OF VIRUS DISEASES IN 1945

Tuber samples were taken from six of the fields included in the survey and were grown in 1946 to given an indication of the spread of virus disease.

Two methods of sampling were used:

A. In three fields, each planted with Scotch certified S.S. seed, 100 tubers were removed at the end of the growing season according to the following plan: during the growing season ten individual plants with leaf roll, at some distance from other leaf roll plants, were marked in each field. Later one tuber was removed from each of the five plants in the row on both sides of the infector. These tubers were labelled 1–5 and 6–10 respectively, and the number in each position which showed leaf roll symptoms in 1946 is given below:

Plant ...	1	2	3	4	5	6	7	8	9	10	Total	Mean no. of infections per infector*
Field 15 (King Edward)	1	2	4	6	9	4	6	1	6	4	43	4·3
Field 18 (King Edward)	6	5	3	5	9	9	6	8	8	5	64	6·4
Field 19 (Red King)	3	2	2	8	7	8	6	4	3	3	46	4·6

* It is recognized that this figure might be too high, since it is possible that infections might have taken place from other infectors.

B. In the other three fields about ½ cwt. of seed-size tubers was taken at random after harvesting and riddling and was grown in 1946. The percentage of virus disease present in these samples is shown below (SM = potato virus *Y* or severe mosaic, LR = leaf roll).

Field 8. Redskin (Sc.) Certificate A. SM 2/210 plants = 0·9 %
LR 30/210 plants = 14·3 %

Field 27. Red King (Sc.) Cert. A. SM 1/462 plants = 0·2 %
LR 139/462 plants = 30·1 %

Field 28. Majestic (Sc.) Cert. H. SM 4/248 plants = 1·6 %
LR 161/248 plants = 64·9 %

No detailed counts of virus disease were made in these fields in 1945, but there were few plants with leaf roll and none was noted with severe mosaic. Thus it would seem that the certificates accompanying the seed gave a true estimate of the virus disease present and that the large amount present in the samples in 1946 was due to spread in 1945. The large differences in percentage between severe mosaic and leaf roll probably reflect the absence of the former from the stock and therefore give no information on any difference in spread of the two diseases.

All six fields were in positions usually considered suitable for potato-seed production, i.e. in exposed positions at altitudes between 800 and 1360 ft. The great spread of leaf roll in 1945 was correlated with a large number of *M. persicae* (over 1000 per 100 lower leaves) which persisted over so long a period (late June to the end of September) in all the fields. Further work will be needed to decide if so large a spread of virus disease is normal or due to exceptional conditions prevailing in 1945.

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The assimilation of ammonium and nitrate nitrogen by detached potato sprouts

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(With 10 Text-figures)

Detached potato sprouts have been grown successfully in ammonium and nitrate culture solutions for 5 weeks. The *p*H and concentration of each solution were such that a similar rate of nitrogen absorption took place from both. The general appearance, increase in fresh and dry weight and the nitrogen distribution in the shoots and roots of sprouts removed at intervals for analysis have been compared. Sprouts, previously grown in nitrate or ammonium culture solutions, have been transferred and grown for 8 days on a minus-nitrogen culture solution, and the changes in appearance, growth rate and nitrogen distribution followed in a similar manner.

The importance of aerating the culture solution, of frequent solution renewal and of an adequate supply of soluble iron for successful solution culture of potato sprouts is stressed.

The ammonium sprouts developed more evenly and ultimately more rapidly than the nitrate sprouts. The nitrate sprouts were a lighter green, showed a tendency for the lower leaves to yellow and dry up and, when transferred to the minus-nitrogen solution, rapidly developed symptoms of nitrogen deficiency. Evidence is presented that, in young potato sprouts, the rate of nitrate reduction and assimilation are a serious limiting factor to the availability of nitrate nitrogen for protein synthesis.

Ammonium assimilation takes place actively and mainly in the roots and stem bases. Nitrate assimilation takes place in both shoot and roots, but is more active in the shoot. Absorption of nitrate ions can take place even when a much higher concentration of nitrate ions is present in the root cells than in the external solution. The initial nitrate nitrogen present in the sprouts is not appreciably utilized during culture on ammonium solution.

The ammonium shoots as compared with the nitrate shoots have a higher content of total nitrogen, protein nitrogen, non-protein organic nitrogen, amide and amino-acid nitrogen, but a lower content of inorganic nitrogen. The nitrate roots as compared with the ammonium roots have a higher content of total nitrogen, protein nitrogen and inorganic nitrogen, but a lower content of non-protein organic nitrogen, amide and other nitrogen.

The higher protein content of the nitrate roots is associated with a higher percentage dry weight and a lower content of 'other' nitrogen. The significance of this relationship is discussed.

During ammonium culture both asparagine and glutamine accumulate, particularly in the shoot.

Evidence is presented that asparagine is the amide primarily concerned in ammonia storage and also that it is formed during nitrate assimilation.

Knowledge of the absorption and assimilation of ammonium and nitrate ions by plants is of fundamental importance for an understanding of fertilizer practice, and the present investigation, in which detached potato sprouts have been successfully grown in ammonium and nitrate culture solutions, forms part of a study of this problem.

Experimental methods

Development of sprouts

Johnston (1919, 1920) sprouted tubers in moist sawdust to develop sprouts suitable for growth in water and sand culture. Mrs N. McDermott (in litt.) found that cutting off a slice $\frac{1}{4}$ to $\frac{1}{2}$ in. in thickness from the heel-end of tubers hastens sprouting.

Seed tubers (King Edward certified—'A' Scot. N.I. 32517) were set to sprout on 14 March 1945. The tubers, trimmed at the heel-end, were placed with their cut surfaces on a $1\frac{1}{2}$ in. layer of moist silver sand in boxes 5 in. deep. The tubers were then buried to a depth of 1 in. in sand and the boxes stored in the greenhouse and watered daily. This treatment was continued for 20 days. The sides of the boxes were then removed, the sand carefully washed away and the sprouts, with their well-developed adventitious roots, excised from the tubers and transferred to running water. Uniformly developed sprouts (average weight 4·32 g.) were selected for the initial analysis and for planting in the water cultures.

Each sprout was carefully freed from superficial moisture by blotting, weighed and immediately transferred to the culture jars. Duplicate sets of plants contained complementary individuals so that at each sampling the effects of cultural conditions

on the development of plants of equal initial weight could be compared. Sprouts similarly prepared and weighed were used for determination of percentage moisture, nitrogen fractions and percentage weight of roots and shoot.

Water-culture technique

Wide-necked glass jars of 800 ml. capacity were used for the culture of single sprouts; stone jars of 7500 ml. capacity were used for the culture of groups of five sprouts. Each glass jar was fitted with a mat of paraffined cork in which had been bored a large central hole to carry the sprout when held in position by a firm plug of non-absorbent cotton-wool and three small holes to carry a thermometer, an aerating tube, and an air exit tube respectively. Each jar was enclosed in a neatly fitting black bag and was shaded from direct sunlight by a specially constructed window box. Each stone jar was fitted with a basket constructed of copper wire coated with bituminous paint. The basket supported a circular paraffined cork mat 2 in. below the upper rim of the jar. Each cork mat carried five large holes for supporting the sprouts. An aerating tube and a level gauge were fitted through the tubulure at the base of each jar. A sleeve of black cloth fitted on to the wire baskets protected the culture solution from light.

Recent work (Arnon & Hoagland, 1944; Clark & Shive, 1932) has emphasized the necessity of adequate aeration in water cultures. The cultures were therefore aerated for three periods of an hour each day. The incoming air passed through a copper cooling coil to a Gallenkamp's rotary blower and vacuum pump driven by a $\frac{1}{8}$ h.p. motor, then through an air-filter and, after saturation with water vapour by passage through a series of wash bottles, into a reservoir fitted with a mercury safety valve. From the reservoir two pipe lines passed to the glass and stone jars respectively. The air supply to each culture was controlled by a screw clip. Each culture was fitted with a glass tube ending in a finely perforated aeration bulb.

Three culture solutions were used: a complete nutrient solution with nitrogen supplied as calcium nitrate; a complete nutrient solution with nitrogen supplied as ammonium sulphate; and a similar solution lacking any nitrogen source. These solutions are referred to as nitrate, ammonium, and minus-nitrogen solutions respectively. The formulae for these solutions are based on those of Arnon (1937) and are set out in Table 1. 0·5 mg. manganese as manganese chloride ($MnCl_2$, $4H_2O$), 0·6 ml. of 0·5% solution of ferrous tartrate, and sufficient 0·1*N* solution of sodium hydroxide (approx. 12·5 ml.) to adjust the *p*H to 6·2 were added to each litre of culture solution before adjustment to volume.

Tiedjens & Blake (1932), using the 4-salt ammonium sulphate culture solutions of Jones & Shive (1923), found that all solutions of *p*H value higher than 6·0 formed some precipitate. Culture solutions, prepared to the formulae shown in Table 1, became cloudy on adjustment to *p*H 6·2, and a slight precipitate settled out on standing overnight. The clear supernatant solution was decanted off and used in the present investigation. All solutions were freshly prepared with distilled water.

Table 1. *Composition of culture solutions*

Salt	Molar (*M*) concentrations		
	Nitrate	Ammonium	Minus-nitrogen
Magnesium sulphate, $MgSO_4$, $7H_2O$	0·003	0·003	0·003
Potassium sulphate, K_2SO_4	0·001	0·0015	0·0015
Calcium acid phosphate, $Ca(H_2PO_4)_2$, H_2O	0·0015	0·002	0·002
Potassium dihydrogen phosphate, KH_2PO_4	0·001	—	—
Calcium sulphate, $CaSO_4$, $2H_2O$	—	0·0015	0·0015
Ammonium sulphate, $(NH_4)_2SO_4$	—	0·002	—
Sodium sulphate, Na_2SO_4, $10H_2O$	—	—	0·002
Calcium nitrate, $CaNO_3$, $4H_2O$	0·002	—	—

In order to maintain the *p*H of the ammonium solutions above 5·8 all culture solutions were renewed frequently; at first every 6 days and then at shorter and shorter intervals until, towards the end of the experimental period, 24-hourly renewals were carried out. No special precautions were taken to maintain sterility in the cultures, but use of a duplicate set of culture vessels made it possible to introduce clean containers at each solution renewal and the cotton-wool plugs holding the sprouts were frequently renewed. Tests for the presence of nitrate in the ammonium solutions using diphenyl-benzidine reagent and for ammonia in the nitrate solution using Nessler's reagent were always negative.

The water-culture equipment was installed in the greenhouse and was in operation from 3 April to 15 May 1945. The average maximum and minimum temperatures during this period were 29 and 11° C. Readings of the temperature of the solution in the

culture vessels were taken each day at 9.0 a.m., 2.0 p.m. and 5.0 p.m. D.B.S.T.; the average temperature at 2.0 p.m. was 20·1° C., the temperature rising from an average of 15·9° C. at 9.0 a.m. to an average of 22° C. at 5.0 p.m.

Methods of analysis

The preparation of the tissue extracts for analysis, the drying conditions used in the determination of the percentage dry weight, and the general methods of estimation of the various nitrogen fractions have been previously described (Street *et al.* 1946).

Total nitrogen. Determination of the total nitrogen by the reduced iron method previously described was found to give variable and low results with the tissues particularly rich in nitrate nitrogen. The method was therefore replaced by a digestion procedure based on that of Doneen (1932). This was found to give consistent results and quantitative recovery of nitrate at a concentration of 0·75 mg. nitrate nitrogen per ml. The sample of powdered dry tissue or the residue obtained by evaporating an aliquot of the aqueous extract to dryness in a rapid current of air at 40° C., was treated in the cold for 40 min. with 2 ml. of N-free sulphuric acid containing 5% w/v A.R. salicylic acid. Then approximately 0·3 g. of A.R. sodium thiosulphate was added and the mixture heated in the Kjeldahl flask until fumes appeared at the neck, cooled and treated with 1 ml. 60% A.R. perchloric acid sp.gr. 1·54 (accurately measured) and 1 drop of saturated copper sulphate solution. This was digested gently until clear and thereafter more vigorously for at least 2 min.

Inorganic nitrogen. The sum of nitrate and ammonia nitrogen is designated inorganic nitrogen.

Non-protein organic nitrogen. The total non-protein nitrogen minus the inorganic nitrogen is designated non-protein organic nitrogen.

Experimental treatments

Series I. A set of twenty-four weighed sprouts, carefully selected for uniformity, were set in the glass culture jars on 3 April. Twelve plants were grown on the nitrate culture solution (nitrate sprouts) and twelve were grown on the ammonium culture solution (ammonium sprouts). Corresponding sprouts from each group were collected after 14 days (five sprouts from each group), 21 days (four sprouts from each group) and 34 days (three sprouts from each group). The sprouts, as collected, were immediately freed from superficial moisture by blotting and weighed. Each sprout was dissected into shoot and roots and these weighed separately. Samples from the bulked shoots and roots of each group were dried for determination of percentage dry weight, and this dry material was used for determination of total nitrogen. The remainder were frozen overnight at −10° C. and extracted for determination of the nitrogen fractions.

Series II. Forty weighed sprouts, selected for uniformity, were set in the stone culture jars on 10 April—twenty sprouts on nitrate and twenty sprouts on ammonium culture solution. On 7 May all sprouts were collected, immediately freed from superficial moisture and weighed. Twelve ammonium sprouts were selected and transferred to the glass jars; six were set in ammonium and six in minus-nitrogen culture solutions (ammonium minus-nitrogen sprouts). Twelve nitrate sprouts were similarly selected and transferred to nitrate and minus-nitrogen culture solutions (nitrate minus-nitrogen sprouts). The remaining ammonium and nitrate sprouts were dissected into shoots and roots, the parts weighed and used for the determination of the nitrogen fractions. After 8 days' growth all four groups of sprouts were collected, blotted and weighed; dissected and the parts weighed; and then the samples used for the dry-weight and nitrogen determination.

Experimental results

Series I. The sprouts remained vigorous and continued to increase in weight during the 5 weeks in culture. The shoots of the ammonium sprouts were distinguishable by their richer green colour and their better proportions. The shoots of the nitrate sprouts tended to elongate excessively and to produce less leaf. Furthermore, after 3 weeks in the nitrate solution some of the lower leaves showed yellowing and those affected quickly dried up and fell off.

The sprouts used had a well-developed root system. This remained healthy and a small amount of new root was produced in both groups, particularly in the ammonium solution. Nevertheless, sloughing of small pieces of root into the culture solution occurred, particularly during the first 14 days, possibly owing to damage in transference to the cultures. The root systems therefore remained fairly constant in fresh weight.

The average increase in fresh weight at each sampling is shown as a percentage of the initial fresh weight (3 April) in Fig. 1. Thirty sprouts selected at random from the population used in this series were each weighed, dissected into shoot and roots and the shoot of each sprout weighed. The shoot was found to constitute 42·4% (standard error ± 0·65) of the fresh weight of the whole sprout. This percentage has been used to calculate the increase in fresh weight of the shoot and roots separately, as shown in Fig. 1.

The average dry weight as a percentage of the fresh weight of shoots and roots is shown for each sampling in Fig. 2.

The course of nitrogen absorption in the nitrate and ammonium cultures, expressed as mg. nitrogen absorbed per g. initial fresh weight of sprouts (3 April weight), is shown in Table 2.

The nitrogen analyses, expressed as mg. N (nitrogen) per g. fresh weight, are shown in Figs. 3–10.

The values for total nitrogen, protein nitrogen, non-protein organic nitrogen and inorganic nitrogen are shown for the ammonium and nitrate shoots in Figs. 3 and 4 respectively and for the ammonium and nitrate roots in Figs. 5 and 6 respectively.

The values for nitrate nitrogen, ammonia nitrogen, glutamine nitrogen, asparagine nitrogen, amino-acid

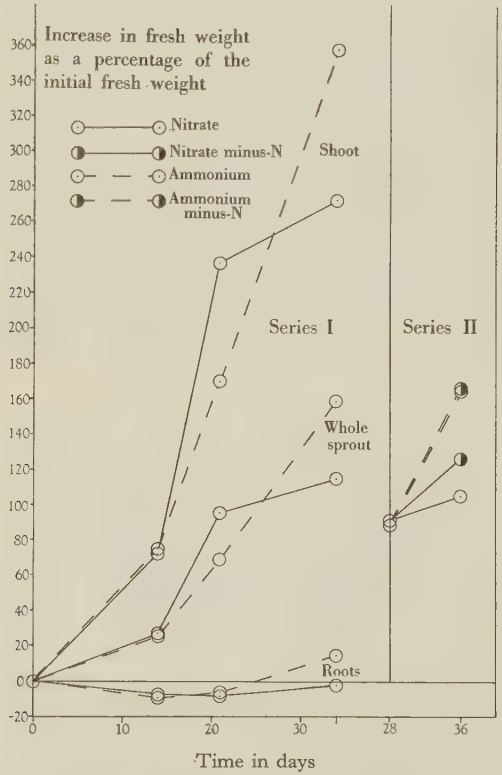

Fig. 1. The average increase in fresh weight of the group of sprouts taken at each sampling, expressed as a percentage of their initial fresh weight. In series I the increases in fresh weight of whole sprouts, shoots and roots are shown separately, the initial weight of shoots and roots being calculated from an analysis of the percentage shoots and roots in the initial sprout population.

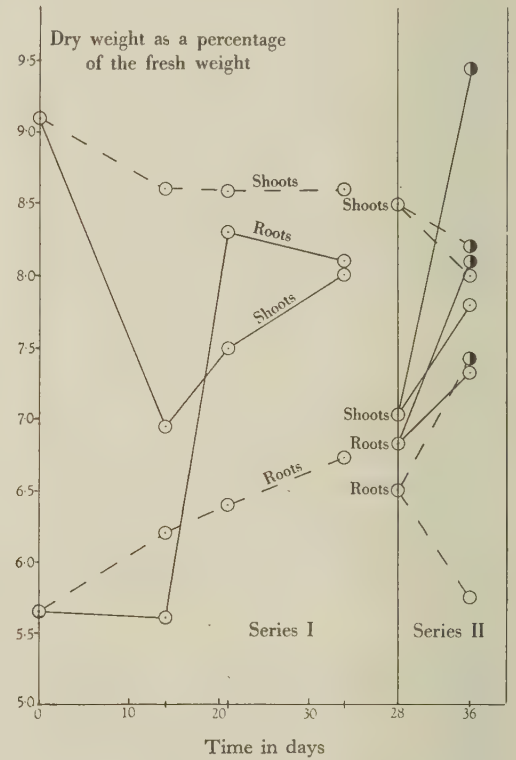

Fig. 2. The average percentage dry weight of shoots and roots at each sampling. Key as shown in Fig. 1.

TABLE 2. *Course of nitrogen absorption in the nitrate and ammonium cultures*

Time in culture (days)	mg. nitrogen absorbed per g. initial fresh weight of sprouts	
	Nitrate cultures	Ammonium cultures
14	1·39	1·54
21	3·86	3·45
34	4·88	7·4

nitrogen and other nitrogen which together constitute the total non-protein nitrogen are shown for ammonium and nitrate shoots in Figs. 7 and 8 respectively, and for ammonium and nitrate roots in Figs. 9 and 10 respectively.

Series II. The ammonium and nitrate sprouts collected from the stone jar cultures on 7 May resembled the typical ammonium and nitrate sprouts described under series I. The nitrate sprouts when transferred to the minus-nitrogen culture solution developed a more general yellowing of the leaves and the lower three or four leaves dried up and fell off during the 8 days' treatment. The ammonia sprouts transferred to minus-nitrogen culture solution lost their typical rich green colour, but no general

yellowing of the leaves developed and only the lowest leaf yellowed and dried up. All sprouts increased in weight and developed new roots. Runners developed from the base of the shoot only in the ammonium minus-nitrogen sprouts.

The average increase in fresh weight of the sprouts is shown as a percentage of their initial fresh weight (7 May) in Fig. 1.

The average dry weight as a percentage of the fresh weight of shoots and roots is shown in Fig. 2.

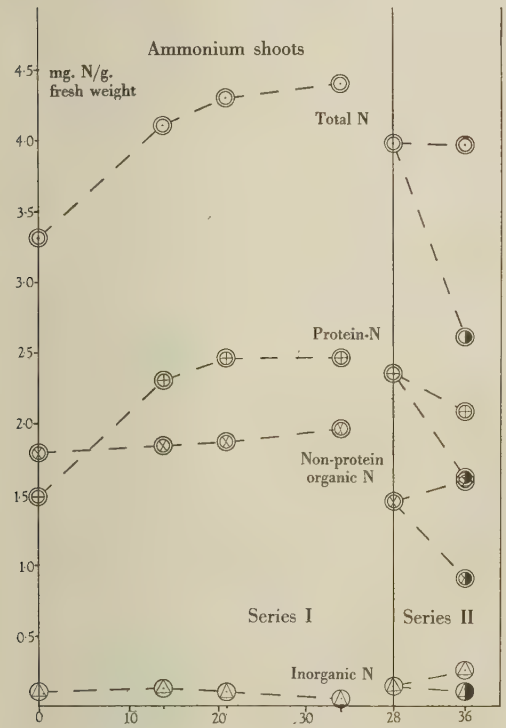

Fig. 3. Content of total nitrogen, protein nitrogen, non-protein organic nitrogen and inorganic nitrogen of the shoots of the ammonium sprouts and of ammonium sprouts grown for 8 days on the minus-nitrogen culture solution. Results expressed as mg. N (nitrogen) per g. fresh weight. Content after the period of minus-nitrogen culture indicated by the half-blacked circles.

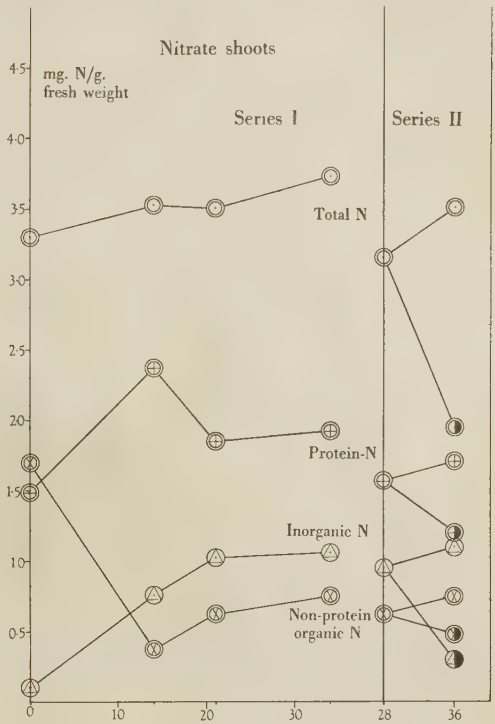

Fig. 4. Content of total nitrogen, protein nitrogen, non-protein organic nitrogen and inorganic nitrogen of the shoots of the nitrate sprouts and of nitrate sprouts grown for 8 days on the minus-nitrogen culture solution. Key as Fig. 3.

The nitrogen analyses are shown along with those of series I in Figs. 3–10. Ammonium and ammonium minus-nitrogen sprouts are shown together in Figs. 3, 5, 7 and 9. Nitrate and nitrate minus-nitrogen sprouts are shown together in Figs. 4, 6, 8 and 10.

Discussion

The rate of absorption and assimilation of the ammonium and nitrate ions is dependent upon their concentration, upon the composition and *p*H of the medium, and upon the species, maturity, physiological vigour and nutritional history of the plant. No attempt has been made in the present investigation to undertake a comprehensive survey of the relative general value of the nitrate and ammonium ions as sources of nitrogen for potato sprouts. The cultures have been conducted at a single *p*H value, and the solutions used have presented the essential elements at a single set of concentrations. The general growth curves therefore apply only to a set of arbitrarily fixed conditions. The choice of *p*H 6·2 was made on the basis of a survey of the data relating to the effect of *p*H on the absorption of nitrate and ammonium nitrogen. The ammonium ion is usually more rapidly absorbed on the alkaline side of neutrality and the nitrate ion on the acid side of neutrality and, in general, the absorption of nitrate is less sensitive to *p*H change than ammonium absorption (Mevius & Engel, 1929; Dikussar, 1929,

1930; Prianischnikow, 1929; Naftel, 1931; Addoms & Mounce, 1932; Hoagland, 1932; and Tiedjens & Blake, 1932). Tiedjens & Blake (1932) found that *p*H values below 6·0 favoured nitrate absorption, and *p*H values above 6·0 favoured ammonium absorption. Pirschle (1929*a*, *b*, 1931) in an extensive investigation of a variety of plants, found that between *p*H 5·5 and 6·5 the difference between plants grown on ammonium nitrogen and those grown on nitrate nitrogen was at a minimum. Tiedjens (unpublished data quoted by Clark & Shive, 1934), has pointed to the possible effect of the *p*H of the culture

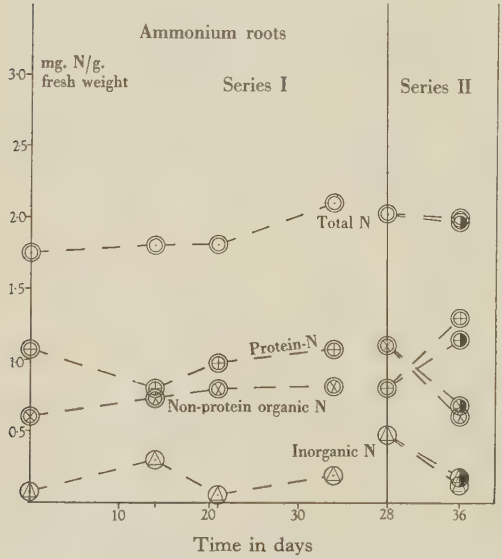

Fig. 5. Content of total nitrogen, protein nitrogen, non-protein organic nitrogen and inorganic nitrogen of the roots of the ammonium sprouts and of ammonium sprouts grown for 8 days on the minus-nitrogen culture solution. Key as Fig. 3.

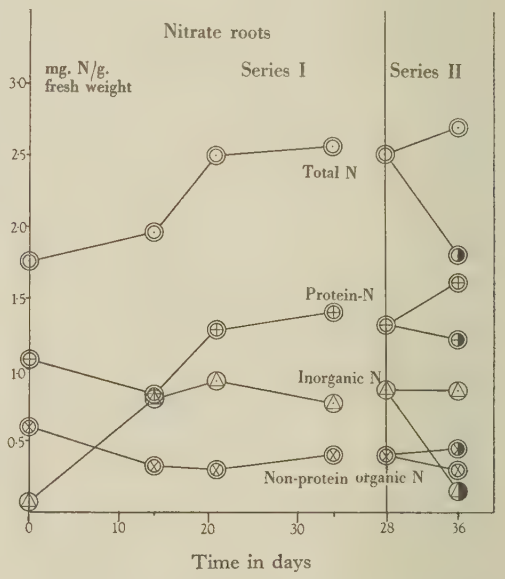

Fig. 6. Content of total nitrogen, protein nitrogen, non-protein organic nitrogen and inorganic nitrogen of the roots of the nitrate sprouts and of nitrate sprouts grown for 8 days on the minus-nitrogen culture solution. Key as Fig. 3.

solution in the dispersion of protein complexes and on the dissociation of soluble ampholytes in the protoplast. He found that the tomato assimilated ammonia nitrogen best when the *p*H value of the culture solution was above that of the plant sap, whereas the reverse was the case with nitrate nitrogen. Hence high *p*H would promote cation combination and low *p*H anion combination. In this connexion is it interesting to note that Robbins (1926) found potato tuber tissue to behave like an ampholyte with an isoelectric point near *p*H 6·4, basic dyes being absorbed and more strongly retained above *p*H 6 and acid dyes being absorbed and more strongly retained below *p*H 6.

The rate of absorption of nitrate is usually more markedly affected by its concentration in the medium than is the case with the ammonium ion (Pirschle, 1931; Tiedjens & Robbins, 1931; Tiedjens & Blake, 1932; and Tiedjens, 1934). Furthermore, there is an increasing tendency for both ions to be equally absorbed at fairly low concentrations. Stewart *et al.* (1925), in experiments with pineapple, found more even absorption at a concentration of 40 mg. N/l. than at 160 mg. N/l. The *p*H (6·2) and concentration (56 mg. N/l.) chosen in the present investigation were therefore designed so that the absorption rates from the nitrate and ammonium solutions would be of the same order. The absorption rates shown in Table 2 closely accord with this expectation. Only after 21 days, when the sprouts are no longer similar in composition or development, does any appreciable difference in the absorption rates become evident.

Very few water- and sand-culture studies of the nutrient requirements of the potato have been undertaken, possibly because its growth habits present certain difficulties for work of this kind. Skinner (1908) has grown detached potato sprouts in aqueous extracts of soil and taken the growth response as a rough index in assessing the suitability of the soils for potato cultivation. The sprouts were grown in jars of small capacity (250 ml.) without aeration or *p*H control. No quantitative data is given, but an examination of photographs shows that root develop-

ment was poor, that most of the lower leaves were shed whilst the sprouts were still quite small and that premature tuber development occurred. Johnston (1919) prepared detached sprouts by a method similar to that adopted in the present investigation, but reported that normal potato plants were not produced either in good garden soil or in nutrient solution from sprouts separated at an early developmental stage from their tubers. The culture solutions used were only renewed every 10 days (capacity of jars about 1 l.), lacked micro-nutrients, were not adjusted in *p*H and were not aerated. Under these conditions the shoots became light green or yellow in colour; the roots were much discoloured and in some cases completely rotted away. The best increases in fresh weight recorded are smaller than those obtained in the present investigation using either the nitrate or ammonium culture solution. Newton (1923) has attempted the water culture of potatoes from tuber pieces (25 g.) with indifferent results. The culture solution (2 l.) was only renewed after 30 days, *p*H is not recorded and no provision was made for aeration. Gericke (1940), however, reports successful growth of potatoes in culture solution on a commercial scale by a general method adopted for other commercial crops.

None of the special difficulties encountered by the earlier workers in water culture of detached sprouts have been evident in the present investigation. The plants remained healthy and developed normally. Only when the nitrate sprouts were transferred to a minus-nitrogen solution did any appreciable yellowing and falling off of the lower leaves occur. It is of interest, however, that the nitrate sprouts were lighter green in colour, and that in the earlier work reviewed not only was nitrogen supplied as nitrate but micro-elements (iron and manganese are probably of greatest importance (Arnon, 1937)) were omitted, and *p*H, with its marked effect on absorption and root development, was not controlled. Although there was little or no increase in the fresh weight of the root system of our sprouts, the roots remained healthy and of good colour and new fine roots were developed. The frequent renewal of the solution, the control of the *p*H and the aeration of the cultures, are

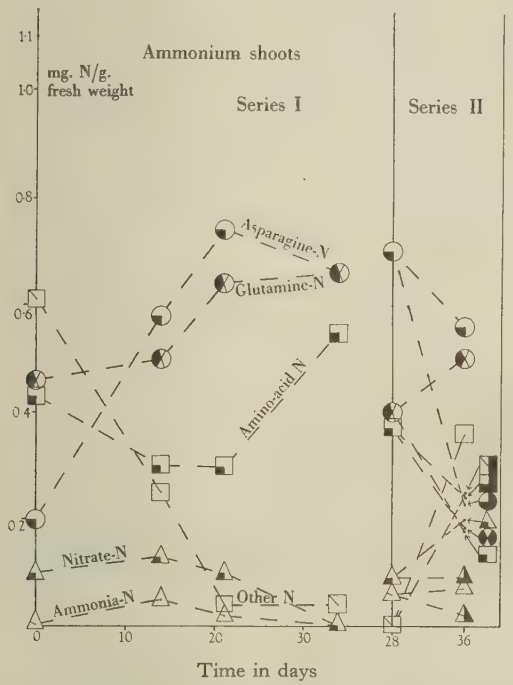

Fig. 7. Content of the separate fractions of the total non-protein nitrogen of the shoots of the ammonium sprouts and of ammonium sprouts grown for 8 days in the minus-nitrogen culture solution. Results expressed as mg. N (nitrogen) per g. fresh weight. Key as Fig. 8.

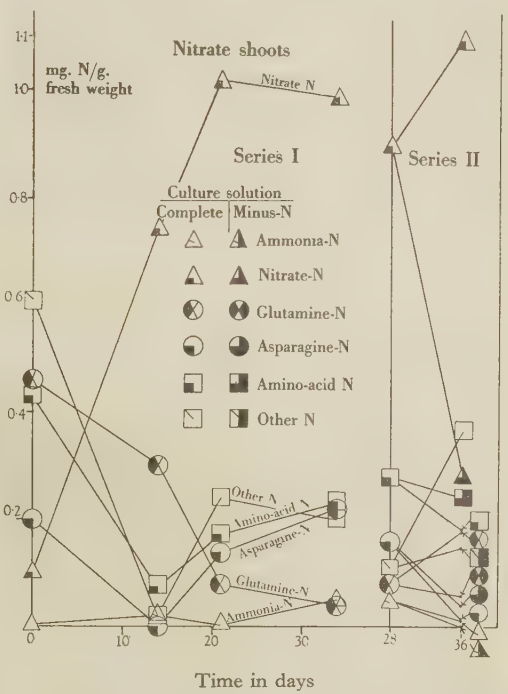

Fig. 8. Content of the separate fractions of the total non-protein nitrogen of the shoots of the nitrate sprouts and of nitrate sprouts grown for 8 days in the minus-nitrogen culture solution.

therefore probably of first importance in maintaining a healthy root system.

Optimum growth of most plants appears to result when nitrogen is available both as ammonium and nitrate, and under these conditions the relative absorption of each ion is a function of the species, its stage of development and of the composition and *p*H of the culture medium. When grown with nitrogen supplied either as ammonium or nitrate many plants seem to show better development with nitrate, but in other cases growth is as good or even superior with ammonium nitrogen. Kruger (1905) reported that the potato grew as well or better with ammonium nitrogen than with nitrate nitrogen, but, as Pardo (1935) has pointed out, the criterion of comparison used is not clearly indicated. The stage of development of the plant is, however, of importance in considering the relative rates of absorption and assimilation of ammonium and nitrate nitrogen. Prince *et al.* (1923) reported a more rapid absorption of the ammonium ion until the plants were 4–5 weeks old, after which the absorption rates were reversed. Naftel (1931), using the cotton plant, and Sessions & Shive (1933) and Stahl & Shive (1933), using oats, have also noted a more rapid absorption of ammonium nitrogen during the early stages of growth. Young seedlings and sprouts probably obtain their initial nitrogen supply as ammonium (Naftel, 1931), or as simple organic nitrogen compounds from the food reserves laid down by the parent plant. Certainly, in the present investigation, the ammonium sprouts developed more evenly and ultimately more rapidly and produced more rich green foliage. The lighter green colour and the tendency for the lower leaves to yellow and dry up, observed in the nitrate sprouts, was suggestive of nitrogen deficiency (Jones & Brown, 1941), particularly as these symptoms rapidly developed in nitrate sprouts transferred to a minus-nitrogen solution. It must be emphasized, however, that the total nitrogen content of the nitrate sprouts never reached a critically low value, and therefore the chlorosis appears to be related either to some other nutrient deficiency or to the availability of the absorbed nitrogen for protein and chlorophyll synthesis. Jones & Shive (1921, 1923) have shown that iron added to cultures as insoluble ferric phosphate is more readily utilized in solutions containing ammonium sulphate than in solutions of the unmodified Tottingham series (1914). Chlorosis was only completely prevented by the ammonium solutions, an effect which was attributed mainly to a lowering of the *p*H at the root surface. The constant renewal of the solution and the presentation of iron as the soluble ferrous tartrate were calculated to prevent iron deficiency in the present cultures. Tiedjens & Blake (1932) have, however, suggested that the ammonium and nitrate radicals may have a specific effect in the availability of iron *within* the plant cell. The contrast between the ammonium and nitrate sprouts may, however, be related to the availabilty of the absorbed nitrogen. Tiedjens &

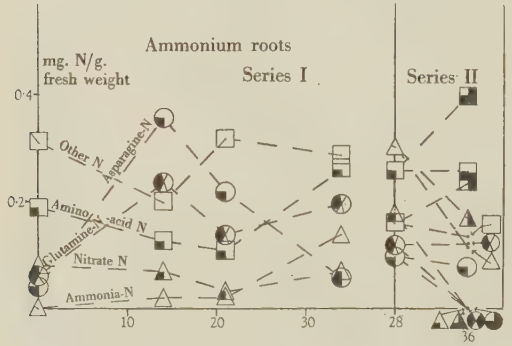

Fig. 9. Content of the separate fractions of the total non-protein nitrogen of the roots of the ammonium sprouts and of ammonium sprouts grown for 8 days on the minus-nitrogen culture solution. Key as Fig. 8.

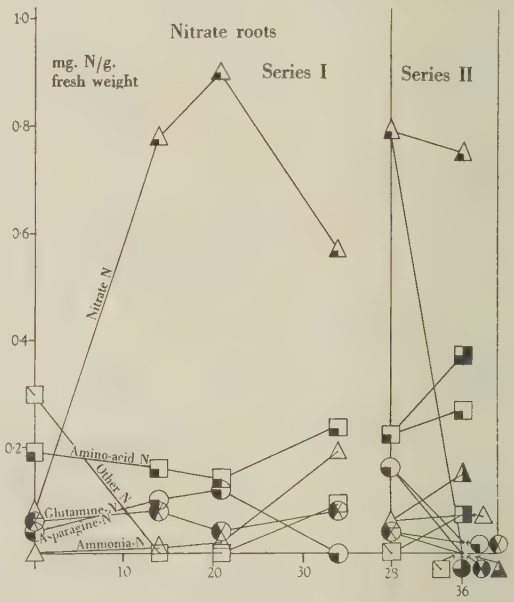

Fig. 10. Content of the separate fractions of the total non-protein nitrogen of the roots of the nitrate sprouts and of nitrate sprouts grown for 8 days in the minus-nitrogen culture solution. Key as Fig. 8.

Blake (1932) have pointed out that when nitrate is taken into the root it will be assimilated in direct proportion to the reductase activity, whilst ammonium will be immediately assimilated, except under conditions of carbohydrate depletion. Nightingale (1937) has noted that a month or more may be required, with no external nitrogen supply, for disappearance of the accumulated nitrate through reduction and assimilation.

Sideris *et al.* (1937, 1938) have confirmed that nitrate nitrogen is usually assimilated more slowly than ammonium nitrogen. Eckerson (1929) considered that the symptoms of nitrogen starvation, which developed with inadequate phosphorus supply, were due to a rapid decrease in reductase activity. The significance of phosphorus as a regulator of reductase activity has, however, been questioned by Breon & Gillam (1944). Davidson & Shive (1934, 1935) have also pointed to the possible influence of the *p*H of the culture solution on the plant's ability to reduce nitrate; nitrate reduction, in the peach trees used, being more rapid at *p*H 4·0 than at *p*H 6·0. Rapid absorption and translocation of nitrate may coincide with development of nitrogen deficiency symptoms if the assimilation rate is critically slow. In the present investigation the nitrate sprouts showed a marked accumulation of nitrate, the shoots containing 0·74–1·10 mg. nitrate nitrogen per g., and the roots 0·57–0·90 mg. nitrate nitrogen per g. No corresponding accumulation of ammonia was observed in the ammonium sprouts, the shoots containing 0·01–0·07 mg. ammonia nitrogen per g. and the roots 0·02–0·31 mg. ammonia nitrogen per g. The data obtained by culture of ammonium and nitrate sprouts on the minus-nitrogen solution (series II) cannot be used to assess the assimilation rate of nitrate by potato sprouts, owing to the loss of tissue of unknown composition by leaf-fall and because no tests were made for loss of nitrogen when the roots, initially rich in nitrate, were cultured in a minus-nitrogen solution. The nitrate sprouts, on transference to the minus-nitrogen solution, increased in fresh weight as rapidly as the ammonium sprouts on either minus-nitrogen or ammonium solution, despite the much greater loss of lower leaves which they suffered. Their total nitrogen content per g. fresh tissue therefore fell below that of normal nitrate sprouts and of ammonium sprouts subjected to the same minus-nitrogen treatment (Figs. 3, 4). Furthermore, at the end of the minus-nitrogen treatment although the protein nitrogen content of the fresh nitrate shoots had fallen from 1·57 to 1·18 mg. protein nitrogen per g. they still contained 0·28 mg. nitrate nitrogen per g. These results therefore indicate that the rate of nitrate assimilation may limit protein synthesis even under conditions where the protein content is at an abnormally low value.

The assimilation of either ammonium or nitrate ions involves utilization of carbohydrates both as the source of the carbon skeleton of the organic nitrogen compounds and as the substrate for the necessary respiratory energy involved in the syntheses. Under conditions of rapid nitrogen assimilation in the dark, carbohydrates are therefore rapidly depleted and there is a loss of dry weight. In the light the dry-weight change will be dependent upon the balance between photosynthesis and respiration. Furthermore, there is evidence that rapid protein synthesis is associated with high respiration rate (Gregory & Richards, 1929; Gregory & Sen, 1937), and, in some cases, it has been possible to demonstrate a correlation between amino-acid content and respiration rate (Spoehr & McGee, 1923). The dry-weight data presented in Fig. 2 may be interpreted on the basis of these considerations. Associated with ammonium culture the moisture content of the roots remains consistently above that of the nitrate roots, possibly owing to accumulation of carbohydrates in the latter. When transferred to the minus-nitrogen solution both roots show an increase in the percentage dry matter, but the effect is much more marked in the ammonium roots. The percentage dry matter of the nitrate shoots fell during the first 14 days of culture, but subsequently increased until the end of the experimental period. Nevertheless, the moisture content remained above that of the ammonium shoots. The percentage dry weight of the ammonium shoots showed a small continuous decrease throughout the experimental period. On transference of ammonium and nitrate sprouts to minus-nitrogen solutions the percentage dry weight of the ammonium shoots continued to decline but less steeply than in the ammonium cultures, whereas with nitrate shoots the normal increase in percentage dry weight was markedly accelerated by the minus-nitrogen treatment. These results are compatible with the view that the ammonium ion is actively and mainly assimilated in the roots and stem bases, and that in the ammonium shoots the utilization of carbohydrates is largely offset by photosynthesis. The rapid rise in percentage dry weight of ammonia roots transferred to minus-nitrogen solution, the occasional presence of an appreciable amount of ammonia in the roots both in the present culture and in previous work (Street *et al.* 1946), the very low concentration of ammonia nitrogen in the shoots, and the accumulation of amides in the stem bases (Street *et al.* 1946) are in agreement with this supposition. Similarly, the evidence suggests that nitrate assimilation takes place in all parts of the plant, but is, in contrast to ammonium assimilation, most active in the shoot system where in consequence carbohydrate accumulation is less pronounced. This view is supported by the observed accumulation of nitrate in both root and shoot, and

the existence of a negative gradient of nitrate nitrogen from leaves to stem bases (Street *et al.* 1946). The initial fall in the percentage dry weight of the nitrate shoots was associated with a rapid utilization for protein synthesis of the non-protein organic nitrogen initially present in the sprouts. In many plants the roots are the chief organs concerned with the transformation of nitrate to organic nitrogen (Davies, 1926; Nightingale & Schermerhorn, 1928; Thomas, 1927; Nightingale & Robbins, 1928; Sani, 1929; Dittrich, 1931; Tiedjens & Robbins, 1931; Tiedjens, 1934). In other cases, however (Eckerson, 1924, 1932; Nightingale & Farnham, 1936; Sideris *et al.* 1938), the tops rather than the roots play the dominant role in nitrate assimilation. Sideris *et al.* (1938), in their work on pineapple, concluded that ammonia was assimilated in the roots as rapidly as it was absorbed, but that very little nitrate assimilation took place in the roots, the greater part being translocated to the chlorophyllous tissues and there utilized in synthesis. The data now presented for young potato sprouts may be similarly interpreted.

The ammonium and nitrate sprouts show well-marked and characteristic differences in their nitrogenous composition. The ammonium shoots are higher in total nitrogen, protein nitrogen, non-protein organic nitrogen, amide and amino-acid nitrogen than the nitrate shoots. The most characteristic feature of the ammonium shoots is their high content of non-protein organic nitrogen. Only in the nitrate shoots does the inorganic nitrogen constitute a considerable fraction of the total nitrogen (Figs. 3, 4). The nitrate roots have a higher total nitrogen, protein nitrogen and inorganic nitrogen content than the ammonium roots. By contrast their content of non-protein organic nitrogen, amide nitrogen and 'other nitrogen' is lower than in the ammonium roots (Figs. 5, 6). This general picture of the contrasting composition of the ammonium and nitrate plants is in broad agreement with that built up by various investigators from similar studies of other plants.

The higher value recorded for protein nitrogen in the nitrate roots is in agreement with the findings of Sideris *et al.* using *Pandanus Veitchii* (1937) and pineapple (1938). These authors have tentatively suggested that because the rate of amino-acid synthesis in the nitrate roots is comparatively slow, carbohydrates are abundant and therefore protein synthesis proceeds continuously, whereas in ammonium roots the rapid conversion of ammonium into simpler organic nitrogen compounds depletes carbohydrate and hence decreases the rate of protein synthesis. Barton-Wright & McBain (1933), in their work on potato, obtained data suggesting that protein can be synthesized from nitrate by direct conversion of the latter into proteose and then into protein. Dikussar (1935) has noted the higher proportion of protein in plants receiving only a limited supply of nitrogen. Fagan (1925), studying the changes which occur during the development of potato tubers, noted that the protein content rises as the moisture content declines, so that a high percentage dry weight is associated with a high-protein content, a conclusion in agreement with our analyses and dry-weight determinations for both the roots and shoots of potato sprouts. It will also be noted that in both nitrate shoots and roots protein nitrogen forms a higher proportion of the total organic nitrogen than in the ammonium sprouts (Figs. 3, 4, 5 and 6).

The non-protein organic nitrogen forms a higher percentage of the total nitrogen in the ammonium than in the nitrate sprouts, the respective values after 34 days' culture being for ammonium shoots 43·5%, for nitrate shoots 20·2%, for ammonium roots 38·8% and for nitrate roots 16%.

The ammonium sprouts did not show accumulation of ammonia nitrogen, except to a very limited extent in the roots, the ammonium ion being assimilated as absorbed. Only in one instance (ammonium shoots, series II) was a slight increase in the low initial nitrate content of the sprouts noted during ammonium culture. This is possibly to be explained on the basis of absorption following the onset of nitrification in the culture solution rather than as an example of the nitrate synthesis observed by Vickery *et al.* (1933) in detached tobacco leaves. The initially low nitrate content of the sprouts otherwise declined steadily during the period of ammonium culture apparently mainly as a result of the increase in fresh weight rather than of assimilation. This is in agreement with the observation of Tiedjens & Blake (1932) that reductase activity was found to be entirely wanting or relatively weak in rapidly growing ammonium-supplied apple trees.

The accumulation of nitrate in both shoot and roots of the nitrate sprouts proceeded until nitrate nitrogen in the shoot reached 29% and in the roots 36% of the total nitrogen. The concentration of nitrate in the root cells must therefore reach a value approximating to 16 times that in the external solution without seriously impairing absorption (Fig. 10).

The amino-acid nitrogen content of the ammonium shoots is higher than that of the nitrate shoots, but there is no significant difference in the content of this fraction in the corresponding roots. The minus-nitrogen treatment led to accumulation of amino-acids in the roots. Since this coincided, in the nitrate roots, with a decrease in nitrate nitrogen and, in the ammonium roots, with a decrease in amide nitrogen, the amino-acid nitrogen became the largest single fraction of the non-protein nitrogen. Before the minus-nitrogen treatment, amino-acid nitrogen constituted 21 and 18·3% of the non-protein nitrogen of ammonium and nitrate roots

respectively; during the minus-nitrogen treatment the value rose respectively to 50·5 and 62·7% (Figs. 9, 10).

The initial sprouts contained, per g. fresh weight, in the shoots and roots respectively 0·46 and 0·06 mg. glutamine nitrogen and 0·20 and 0·04 mg. asparagine nitrogen. During culture in nitrate solution the glutamine nitrogen content of the shoot declines to 0·04 mg./g., whilst in the root the initially low value was maintained. The asparagine nitrogen content of both shoot and root varied during culture in nitrate solution, sometimes disappearing completely and on other occasions rising considerably above the initial value. Sideris *et al.* (1938) concluded that, in pineapple, nitrate appeared to be converted, in the leaves, into various forms of soluble organic nitrogen, especially asparagine and basic amino-nitrogen. The behaviour of the nitrate sprouts can similarly be interpreted as showing a gradual depletion of the reserve of glutamine present in the shoots, concomitant with varying synthesis and depletion of asparagine in both shoot and roots (Figs. 8, 10). During culture in ammonium solution accumulation of both amides occurred, particularly in the shoots, but here again a fluctuating level of asparagine nitrogen was noted. Glutamine is normally present in greater concentration than asparagine in potato tops (Street *et al.* 1946), and this was the case in the sprouts used in the present investigation. On culture in ammonium solution, however, although both amides accumulated, the increase in asparagine was more pronounced, so that in the ammonium shoots it equalled or exceeded the glutamine (Fig. 7). On transference of either ammonium or nitrate sprouts to the minus-nitrogen solution, the content of both amides decreased, the asparagine being utilized more rapidly. After the treatment amides were only present in traces in the nitrate shoots and were absent from both nitrate and ammonium roots (Figs. 7, 8, 9 and 10). The evidence in favour of asparagine synthesis in nitrate-fed sprouts and its marked accumulation under conditions of ammonium nutrition suggest that in potato asparagine is the amide most directly concerned in ammonia detoxication and storage. These data in so far as they expose a difference in behaviour of the two amides and relate asparagine more intimately with ammonia storage do not conflict with the view, tentatively discussed elsewhere (Steward & Street, 1946), that glutamine plays an important role in the mechanism of protein synthesis.

The 'other nitrogen' values are obtained by difference and therefore should be interpreted with caution. The 'other nitrogen' content of both ammonium and nitrate shoots varied from 0·02 to 0·37 mg. nitrogen per g. but appeared to fluctuate irregularly within these limits (Figs. 7, 8). The roots, however, are clearly contrasted in their 'other nitrogen' content, this fraction accounting for 0·13-0·32 mg. nitrogen per g. in the ammonium roots, but being almost completely absent from the nitrate roots (Fig. 10). Tiedjens & Blake (1932), using apple trees, have also recorded higher values for 'other nitrogen' in their ammonium plants. The 'other nitrogen' includes peptide and polypeptide nitrogen, and it appears that the higher values for this fraction in the ammonium roots are connected with their higher water content and lower content of insoluble protein.

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The formation of insecticidal films on building materials

I. Preliminary experiments with films of pyrethrum and D.D.T. in a heavy oil

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The relative toxicity to the flour beetle, *Tribolium castaneum* Herbst, of films of 1·6% w/v total pyrethrins or 0·8% w/v technical D.D.T. in Shell Oil P31 sprayed on to some common building materials has been investigated. Beetles were exposed on films for 6 days, starting 24 hr. after application of the insecticide, and relatively low kills were recorded even with deposits two to three times as heavy as those normally used in practice. The materials could be placed in order, according to the toxicities of the films formed on them, as follows:

1·6% pyrethrins	0·8% D.D.T.	
(1) Rough deal, sacking	Rough deal, limewashed brick	Films more toxic
(2) Fletton brick	Fletton brick	↓
(3) Wallboard		
(4) Cement, cement-sand, limewashed brick, ceiling board	Cement, cement-sand	Films less toxic

An experiment carried out under practical conditions confirmed the low toxicities of films formed on limewash and wood.

The effect has also been investigated of pretreating bricks with six substances, which might influence the rate of sorption of the oily insecticide into the substrate. Pretreatment with polyvinyl alcohol, starch paste, size, and waterglass greatly increased the toxicity of pyrethrum films on brick, but water and calcium chloride failed to do so. The toxicity of a pyrethrum film on cement-sand blocks was also very greatly increased by a pretreatment with starch paste.

Introduction

During the application of sprays in warehouses for the control of insects infesting stored produce, a considerable proportion of the population is not affected by the insecticide, since the insects are out of reach in stored goods or in crevices in the fabric of the building. If, therefore, a toxic film of insecticide could be made to persist for several days or longer on the surfaces over which these insects might subsequently wander, the value of the spraying treatment should be greatly enhanced.

The use of films of pyrethrum in a refined medium-heavy white mineral oil of relatively low vapour pressure to control the moths and larvae of *Plodia interpunctella* Hb. and *Ephestia elutella* Hb., infesting boxes of dried fruit, has been described by Potter (1935, 1938). The same author (1938) has given an

account of preliminary experiments to assess the toxicity of such films to other insects. Since that time, pyrethrum films have been applied in warehouses for treating residual infestations by a relatively wide range of insect species, particularly under the abnormal conditions of storage of grain and other foodstuffs during the war, in the hope that effective insecticidal films would be produced on the different building surfaces.

Some exploratory experiments carried out at the Pest Infestation Laboratory about 6 years ago indicated that films sufficiently toxic to kill the granary weevil, *Calandra granaria* L., were not readily formed on surfaces common in buildings. Field observations subsequently supported this conclusion. The short effective life of the films was thought to be due mainly to the relatively rapid sorption into most building materials of the insecticide, which thus became no longer fully available for transference to the insects. The present investigation was therefore started in order to gain some knowledge of the factors influencing film formation, persistence, and toxicity with the ultimate object of improving the persistence of toxic films and economizing in the use of insecticides.

Laboratory investigation of the relative toxicities of films on building materials

Experimental samples

The following were chosen to include materials comparable to those found in warehouses.

(*a*) *Bricks.* Fletton type bricks, taken from a pile in the open air and therefore not conditioned in any way, were each divided into three parts about $3 \times 4\frac{1}{2} \times 2\frac{1}{2}$ in. Samples for spraying were taken at random in all but the pretreatment experiments, in which films on the uncut surfaces of the three parts of the same brick were compared.

(*b*) *Wood.* Planed deal samples, $2\frac{1}{2} \times 2\frac{1}{2} \times \frac{3}{4}$ in., were used in the earlier experiments, but subsequently pieces of well-seasoned rough deal, $3 \times 3 \times 1$ in., were employed.

(*c*) *Cement.* Portland cement samples, $3 \times 3 \times \frac{1}{2}$ in., were cast in wooden moulds and then dried for 24 hr. over a radiator.

(*d*) *Cement-sand mixture.* Samples, $3 \times 3 \times \frac{1}{2}$ in., were prepared containing 1 part of cement to 4 parts of sharp sand by volume and dried as in (*c*). This cement-sand mixture was roughly comparable to concrete: it was impracticable to make concrete samples of such small size.

(*e*) *Limewash.* An aqueous suspension of slaked lime was applied by brush in two coats to brick samples and allowed to dry in the laboratory for 4 days before spraying with insecticide.

(*f*) *Fibre board.* Samples, $3 \times 3 \times \frac{1}{2}$ in., were cut from a board consisting of compressed wood fibres.

(*g*) *Ceiling board.* Samples, $3 \times 3 \times \frac{3}{16}$ in., were cut from a board consisting of wood fibres in a plaster matrix.

(*h*) *Sacking.* Circular samples, 7 cm. in diameter, were cut from closely woven grain sacking.

The surfaces of (*c*) and (*d*) in contact with the mould or the air when setting are referred to respectively as 'moulded' and 'free'. The unpatterned surfaces of (*f*) and (*g*) were sprayed. The toxicity of films formed upon sacking is important in view of the frequent occurrence of sacks in warehouses.

Experimental technique

The insecticides used were solutions of 1·6% w/v total pyrethrins in Shell oil P31, and 0·8% crude D.D.T. in P31. The crude D.D.T. contained 74·6% of the *p*, *p'*-compound. Using the film technique of Parkin & Green (1943), 0·8% D.D.T. was found equivalent to 1·6% pyrethrins.

One surface of each sample was sprayed at 15–20° C. with insecticide in a spraying apparatus similar in essentials to that described by Potter (1941). Within 15 min. the samples were transferred to a room maintained at 25° C. and 70% R.H., where they remained until the end of the experiment. The weights of the deposits of insecticide could not be found directly, because of the relatively large masses of the samples, but were estimated by first spraying a cover-slip of known area. When the required deposit had been obtained on cover-slips on three successive occasions, not more than four samples were sprayed by atomizing the same quantity of insecticide each time: the working of the apparatus was then checked by another cover-slip test. The deposits on the groups of samples were assumed to be the same as those on the corresponding cover-slips and were varied from 0·5 to 4·0 mg./sq.cm. (approx.).* The deposits generally used in practice are of the order 1–1·5 mg./sq.cm.

Adults of the flour beetle, *Tribolium castaneum* Herbst, were used as the test insect, and were bred and handled as described by Parkin & Green (1943). Fifty beetles were confined within a glass ring, 6 cm. in diameter and 2 cm. in height, sealed to the somewhat irregular surfaces of each sample with an external ring of plasticine.

The numbers of beetles dead and knocked down were counted after exposure on the films for 6 and 9 days. An insect was considered dead if it neither moved spontaneously nor responded by reflex movement on slight pressure with a soft brush. An insect was counted as knocked down if it lay on its back, unable to regain the normal position: knock-down in this sense included death.

* More recent experiments have indicated that the deposits on the relatively rough surfaces of the samples were some 10% higher than on the smooth surface of the glass cover-slips.

Results

Preliminary experiments with planed deal showed that, immediately after spraying, there was free insecticide on the surface and that this was no longer visible after a few hours. It was thought, therefore, that confinement of the insects on the film directly after spraying would not test satisfactorily the properties of the material in relation to film persistence. Tests were carried out on planed deal, delaying the application of beetles for 0, 2, 6 and 24 hr. after deposition of about 2 mg./sq.cm. of pyrethrum solution. The toxicity of the film fell considerably during the first 6 hr., but scarcely noticeably between 6 and 24 hr. after spraying. Films on rough deal showed a similar but less pronounced effect. As judged by eye, the two deals were the least absorbent surfaces used, the smooth less so than the rough. In view of these findings a 24 hr. delay in putting on insects was adopted for all surfaces in subsequent tests.

Pyrethrum films covering a range of deposits were sprayed on to rough deal, brick, and cement-sand mixture. Twenty-four hours later batches of beetles were confined on the films and the numbers dead counted after 6 days' exposure. The data are summarized in Table 1.

The heterogeneity shown by these data was not unexpected in view of the probable variation between the samples. Films on rough deal and brick were clearly more toxic than those on cement-sand mixture, which failed to support a film toxic to *T. castaneum* even at the highest level of deposit. On deal and brick the toxicity increased with increasing deposit, as might have been expected.

In view of these results it was decided to employ deposits of about 4 mg./sq.cm. in subsequent comparisons of surfaces. A further set of tests was carried out, the results of which are given in Table 2.

The data confirmed that pyrethrum films formed on wood and brick were more toxic than those on cement-sand, with which could also be grouped cement alone and limewash on brick. These results showed more clearly than those of Table 1 that rough deal was superior to bricks as a substrate for films of pyrethrum in a heavy oil. It should be noted that the knock-down was very much higher on the first two than on the last three treated surfaces listed in the table.

In tests with pyrethrum films on other materials, and in tests with D.D.T. films, deposits of about 4 mg./sq.cm. were employed as before. The results, in conjunction with those in Table 2, may be summarized as follows:

1·6 % pyrethrins

(1) Rough deal, sacking
(2) Fletton brick
(3) Wallboard
(4) Cement, cement-sand, limewashed brick, ceiling board

0·8 % D.D.T.

Rough deal, limewashed brick — Films more toxic
Fletton brick
↓
Cement, cement-sand — Films less toxic

The relative toxicities of the films formed on the surfaces of groups (1)–(4) may be judged from Table 2, with the additional information that on wall-board pyrethrum films knocked down about 40 % of the insects and killed 6–10 % in 6 days.

TABLE 1. *Mortality of adult* Tribolium castaneum *after 6 days' exposure at 25° C. and 70 % R.H. on pyrethrum-in-oil films deposited on three building materials*

Estimated deposit (mg./sq.cm.)	Mortality (%) Rough deal	Brick	Cement-sand
0·52–0·57	6, 12*	10, 16	0, 2
1·00–1·03	22, 32	16, 36	0, 0
2·03–2·08	25, 40	17, 22	0, 4
3·97–4·05	40, 44	22, 50	0, 2
Control	2	0	4

* Two replicates for each deposit and surface.

TABLE 2. *Additional tests of the toxicity to adult* Tribolium castaneum *of pyrethrum-in-oil films deposited on various materials*

Estimated deposit: 4·00–4·10 mg./sq.cm.
Counts made after exposure for 6 days.

	No. of insects exposed	Mortality %	Knock-down %
Rough deal	200	64–80	100
Brick	200	18–34	100
Cement-sand*	200	12–22	12–22
Cement*	200	4–14	4–14
Limewash on brick	200	6–12	6–12
Controls (all surfaces)	350	0–12	0–12

* Both moulded and free surfaces used.

A COMPARISON UNDER PRACTICAL CONDITIONS OF THE TOXICITY OF FILMS FORMED ON WOOD AND LIMEWASHED BRICK

In view of the generally low toxicity of the films formed in the laboratory on certain surfaces, an experiment was carried out in a warehouse in London to discover whether the results obtained under practical conditions would be similar to those obtained in laboratory tests. This experiment was essentially exploratory, since there was no past experience from which to decide the most suitable method of exposing the insects to the films or the best way of assessing the results.

Experimental technique

The general plan of the experiment was to spray areas of a wooden floor and a limewashed brick wall with pyrethrum or D.D.T., and to confine insects on each treated surface at different times after spraying. For controls, insects were confined on unsprayed parts of the surfaces. After exposure, the insects were transferred to a constant temperature room (25° C.) at the laboratory. Observations were made to assess the toxicity of the films to the insects, both on the films and at intervals after removal to constant temperature.

The insecticides used were 1·6 % w/v pyrethrins and 0·5 % crude D.D.T. in P 31. These were sprayed as in general warehouse spraying practice, by means of a DeVilbiss oil-spray gun working at an air pressure of 65–70 lb./sq.in. The deposit of insecticide on the floor was measured by exposing weighed filter papers to the spray, and weighing these again after spraying. The deposits were determined as 0·4–0·6 mg./sq.cm.

The test insects were *T. castaneum*, similar to those used in laboratory tests, but conditioned for 4 days at 10–12° C. before exposure on the films. Batches of twenty insects were confined on the wall by funnels, 6 cm. in maximum diameter, while batches of fifty insects were confined on the floor by the same method as in laboratory experiments. In each exposure period three batches of insects were exposed on the wall and on the floor in each of the three experimental areas, viz. one sprayed with pyrethrum, one sprayed with D.D.T., and a control area. After exposure on the films the insects were removed to a constant-temperature room at the laboratory maintained at 25° C. and 70 % R.H.

It was decided initially to leave all the insects on the films for 3 days before removing them to constant temperature, but in view of the low mortalities in the first batches of insects, the second and third exposure periods were extended to 6 days. The exposures overlapped as follows:

Exposure	Initial age of film	Exposure period
First	1–2 hr.	3 days (1st–4th days)
Second	24 hr.	6 days (2nd–8th days)
Third	4 days	6 days (5th–11th days)

The numbers of insects knocked down were observed *in situ* after the insects had been exposed on the films for 3 and 6 days. Subsequently, knock-down and mortality were counted when the insects had been kept 3 hr., 3 days, 6 days and 9 days at 25° C.

A thermohygrograph was kept in the warehouse during exposure of the insects. The temperature was 10–14° C., and the relative humidity 80–93 %, except during the last 24 hr., when the humidity dropped to 70 %. Apart from the last 24 hr., changes were gradual, especially those of temperature.

Results

The large body of data obtained was highly consistent, and only a portion of it is summarized below. Tables 3 and 4 give respectively the mean percentage knock-down of the beetles after exposure on the film for 3 days, and mean percentage mortality after storage at 25° C. for 9 days.

TABLE 3. *Mean percentage knock-down of* Tribolium castaneum, *after exposure on insecticidal films for 3 days under practical conditions*

		Initial film age		
Insecticide	Surface	1–2 hr.	24 hr.	4 days
Pyrethrum	Limewash	< 50	2	0, 25, 90*
	Wood	100	100	100
D.D.T.	Limewash	0	0	0
	Wood	13	12	5
Control	Limewash	0	1	0
	Wood	3	0	0

* In view of heterogeneity the figures for separate replicates are given.

TABLE 4. *Mean percentage mortality of* Tribolium castaneum *9 days after removal from insecticidal films to 25° C. and 70 % R.H.*

		Exposure		
Insecticide	Surface	1st	2nd	3rd
Pyethrum	Limewash	3	18	20
	Wood	25	48	32
D.D.T.	Limewash	8	6	4
	Wood	8	26	13
Control	Limewash	3	9	5
	Wood	11	6	4

The generally low values for knock-down and kill in Tables 3 and 4 show clearly that toxic films of poor persistence are likely to be formed in practice and confirm the conclusions drawn from the laboratory experiments.

In the warehouse, the films formed by both pyrethrum and D.D.T. on wood were more toxic than those on limewash. This result agrees with that of the laboratory experiments with pyrethrum but not with D.D.T. for, in the latter, films of D.D.T. on limewash and on wood were approximately equal in toxicity. This discrepancy may be due partly to the use of a lower concentration of D.D.T. in the warehouse experiment than in the laboratory tests. However, in addition, the limewash coating employed in the laboratory was much more friable, owing to the absence of binding material, than that in the warehouse. For this reason, in the laboratory experiments the insects became coated with lime

particles, whereas in the warehouse they did not, so that the insecticide was likely to have been more effectively transferred to the insects under the former conditions. This particular laboratory result should therefore be accepted with reserve.

Preliminary experiments on the toxicity of pyrethrum films on pretreated surfaces

The relatively low toxicities of pyrethrum films formed on surfaces such as limewash, cement, etc., indicated that, under practical conditions, effective films could not be expected on the materials investigated, with the possible exception of wood and sacking. Since the lack of toxicity must be due to a physical or chemical interaction of insecticide and substrate, attempts have been made to prevent this interaction by pretreating the surface of the substrate and thus interposing a thin layer of oil-impermeable material between the two phases.

Experimental technique

Bricks were used as the substrate for the first trials of pretreatments, because of the intermediate toxicity of films of pyrethrum in oil deposited upon them. Four bricks were each split transversely into three approximately equal pieces. One piece from each brick was sprayed with 1·6% pyrethrins in P31, as in other laboratory experiments, without pretreatment. The two remaining sets of samples were pretreated respectively with two of the substances under investigation, dried thoroughly, and then sprayed with insecticide. In this way, each group of four bricks sufficed for the evaluation of two forms of pretreatment, using four replicates on matched samples.

Seven pretreatments were tested:

(1) 5% polyvinyl alcohol in water, applied with a brush as a single coat.

(2) Starch paste (a proprietary preparation for office use) used as supplied and applied as (1).

(3) Size, a viscous solution in water, applied as (1).

(4) Waterglass (a proprietary brand of egg preservative) used undiluted and applied as (1).

(5) A small amount of water applied by brush immediately before spraying.

(6) A larger amount of water applied by immersing the surface, which was later to be sprayed, in distilled water for 1 hr., by which time the water had penetrated 1–1½ in. into the brick. The samples were removed from the water just before spraying and the free water wiped off with a clean cloth.

(7) 10% aqueous calcium chloride solution brushed on and the treated surfaces sprayed with insecticide 16 hr. later.

Table 5. *The toxicity to adult* Tribolium castaneum *of films of pyrethrum in oil on pretreated bricks*

Estimated deposits: 3·98–4·06 mg./sq.cm. of 1·6% pyrethrins. Counts made after exposure period of 6 days on films 24 hr. old at beginning of exposure.

Surfaces not pretreated				Surfaces pretreated		
Sprayed		Unsprayed		Sprayed		Unsprayed
Knock-down %	Kill %	Kill %	Pretreatment	Knock-down %	Kill %	Kill %
98–100	2–24	2–10	Polyvinyl alcohol	98–100	34–100	0–2
100	8–24	10	Starch paste	100	90–94	4
100	2–51	2	Size	100	100	2
100	2–51	2	Waterglass	100	100	2
100	6–14	0	Water (light application)	100	2–8	4
98–100	2–10	0	Water (heavy application)	12–75	2–6	4
100	6–14	0	10% $CaCl_2$	100	0–6	2
			Appendix: Cement-sand pretreated with starch paste			
8–22	8–22	26	Starch paste	100	100	8

Results

The results of the comparison are given in Table 5. These results show that pretreatment with polyvinyl alcohol, starch paste, size and undiluted waterglass increased the toxicity of the insecticidal film. Water and the hygroscopic calcium chloride, however, failed to do this; in fact, from the knock-down figures, it appears that the heavy application of water actually reduced the toxicity of the film formed subsequently.

In order to discover whether one of the successful pretreatments would act on a surface less favourable to film formation than brick, starch paste was brushed on to cement-sand samples. The results of this test are given in the appendix to Table 3. The starch

paste increased the toxicity of the film formed, and there seems no reason to doubt this result in spite of the high kill on the untreated cement-sand.

Discussion

The deposits of insecticide used to produce toxic films on the building surfaces tested in the laboratory were $2\frac{1}{2}$–3 times as heavy as those normally applied in practice. It is therefore probable that, 24 hr. or less after application of the spray to some of the commoner building surfaces in warehouses, there remains no insecticidal film of sufficient potency to kill relatively resistant insects like adult *T. castaneum*.

The different toxicities of the films formed on the non-pretreated materials cannot be accounted for without further experimental work. It is likely, however, that the toxicity of the films depended upon at least two factors, viz. (*a*) the volume of liquid insecticide transferred from the substrate to the cuticle of the insects, and (*b*) the concentration of active principle in (*a*). In turn (*b*) will depend upon (*c*) the rate of decomposition of active principle, and (*d*) the extent of selective adsorption of active principle by the substrate.

Sacking differed from the other materials tested in that droplets of insecticide remained suspended from projecting fibres, so that (*a*) was probably larger for this material than for the rest. Sacking is not likely to contain substances which will decompose pyrethrins, so that (*b*) should not fall rapidly.

For the remaining materials there is no *a priori* reason to suppose that (*a*) was a function of the rate of capillary absorption of the insecticide, since all free insecticide appeared to have left the surfaces before exposure of the insects began. It seems probable that (*a*) was derived from the film remaining at the surface after capillary absorption and redistribution had taken place. There are no data on (*d*) at the moment, but (*c*) in the case of pyrethrum might be connected with alkalinity. Walker (1931) found that the pyrethrins were relatively stable at *p*H 9·5, but decomposed very rapidly at *p*H 12; similarly, Tattersfield & Hobson (1931) showed that a pyrethrum emulsion of *p*H 11 had lost some of its toxicity 1 day after it had been made up. The normal *p*H values for deal, brick, and for the group (4) substrates are respectively 4–5, 7 and 10–12. For pyrethrum, the toxicity of the films was negatively correlated with the *p*H of the material on which the films were formed. Data on the stability of D.D.T. at different *p*H values are not available.

Sufficient data have been given to show that a great increase in persistence of toxicity of insecticidal films can be achieved if absorbent and/or alkaline surfaces are suitably pretreated. Of the pretreatments tried polyvinyl alcohol, starch paste, size, and waterglass were obviously effective. It was evident by visual inspection that polyvinyl alcohol and starch paste slowed down the rate of absorption of the spray; but by the time the insects were put on to the films, all free spray had been absorbed, at least so far as could be detected by wiping the surfaces with a finger. For these two pretreatments, therefore, it appears that, although the spray may be absorbed, it is held more readily available to the insects than on an untreated surface. In the cases of waterglass and size, on the other hand, free insecticide remained upon the surfaces until the end of the exposure period (i.e. 10 days after spraying).

The failure of water and calcium chloride solution to increase the persistence of the oily films was interesting. While there are theoretical indications that water would not necessarily decrease the rate of capillary absorption of pyrethrum in oil, the subject is a complicated one, and further experimental work is required before it can be fully discussed.

The great advantages to be gained from the use of some form of pretreatment are evident, provided that a suitable substance can be discovered which has no serious disadvantages in availability, cost, ease of application, etc. A wider range of substances has therefore been examined and data upon their efficacy will be presented in a later paper.

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A note on the efficiency of a pyrethrum spray in controlling *Ephestia elutella* Hb. moths in a granary

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A stowage in a London granary containing 500 tons of Manitoba wheat was sprayed with 0·8% w/v pyrethrins in Shell oil P31, in an attempt to control an *Ephestia elutella* infestation. The mean deposit on the surface of the wheat was 1·5 mg./sq.cm., of which one-sixth was due to the settling of mist.

All the moths in the stowage at the time of spraying, estimated at 68,900, were killed or fatally affected within 24 hr., and those which emerged later, estimated at 7500, were killed by the insecticidal film.

The spray was not observed to have any marked effect on the size of the migrating larval population.

The use of atomized oil insecticides containing pyrethrum for the control of *Ephestia* and *Plodia* infestations in stored food products was first investigated by Potter (1935, 1938). No accurate method of assessing the degree of control achieved was available to Potter, although he was able to obtain some indication of the effectiveness of the treatment by making general observations on the moth population before and after spraying, and comparing the degree of infestation during the year when the insecticide was applied with that of a previous year when no control measure was adopted.

A method for estimating the numbers of moths and larvae in an infestation of *Ephestia elutella* Hb. on bulk wheat has recently been developed by Richards & Waloff (1946), and this method has now been put to practical use in ascertaining the effect on the insect population of an insecticidal treatment of wheat and its stowage.

The first trial of the method took place in 1943, when weekly estimates were made of the number of *E. elutella* moths in a stowage in a London granary. The stowage was twice sprayed with an 0·8% w/v pyrethrum-in-oil spray during the period in which the estimates were made. Table 1 shows the estimated number of moths present at each count. The first spraying took place at, or just before, the time of maximum moth emergence. Although the number of moths in the stowage fell from 27,400 to 600 as a result of the spraying, it rose again 9 days later to 14,300, and to a new maximum of 16,500, 24 days after treatment.

The spraying operation

The experiment now described was carried out in the same granary but in a different stowage, namely, that in which Richards & Waloff undertook their detailed study of an *Ephestia* population, and which, therefore, had received no insecticidal treatment for over 2 years. The stowage contained about 500 tons of Manitoba wheat, and the total area of exposed surfaces (walls, ceiling, pillars and grain) was approximately 12,000 sq.ft.

Table 1. *Estimates of the number of* Ephestia elutella *moths in a stowage in a London granary: June–August* 1943*

Date of count	Estimated no. of moths
15. vi. 43	900
17. vi. 43	2,500
21. vi. 43	4,700
29. vi. 43	27,400
5. vii. 43	Stowage sprayed
6. vii. 43	600
15. vii. 43	14,300
22. vii. 43	15,100
29. vii. 43	16,500
5. viii. 43	9,400
12. viii. 43	3,100
?. viii. 43	Stowage sprayed
19. viii. 43	13
26. viii. 43	8

* Unpublished results of Richards & Waloff.

Spraying was carried out on 20 July 1945, by three operators using DeVilbiss H.M. spray-guns. Compressed air was supplied at 60 lb./sq.in. by a petrol-driven compressor, and the spray consisted of 4 gal. of 0·8% w/v pyrethrins in Shell oil P31, the quantity being calculated on the basis of 1 gal./300 sq.ft. of surface (about 1·4 mg./sq.cm.).

The object of the spraying was to deposit a lethal film of insecticide on all exposed surfaces, and at the same time to obtain the maximum mist effect on flying moths. The actual spraying time was 1 hr. 23 min., but, because of a compressor breakdown, the total time taken was 2 hr. 12 min.

The weight of spray which was deposited on the grain was measured by the use of glass plates 5 cm.

square. The plates were weighed in the laboratory in the bottom portions of Petri dishes, the lids replaced and the dishes plus plates packed for transportation to the warehouse. Here the plates were placed on clean filter-papers on the grain and exposed during and after spraying. Thirty-three plates were exposed during the whole treatment, i.e. to direct spray and mist, while ten plates were exposed to the mist alone. After exposure the plates were carefully replaced in their respective Petri dishes and brought back to the laboratory where they were reweighed. Data on the deposits are shown in Table 2.

TABLE 2. *Summary of data on deposits obtained during spraying on* 20 *July* 1945

	Plates exposed to direct spray plus mist	Plates exposed to mist alone
No. of plates exposed	33	10
Range of deposits (mg./sq.cm.)	0·48–4·42	0·04–0·37
Mean deposit (mg./sq.cm.)	1·5	0·25
Standard error (mg./sq.cm.)	0·16	0·02

It is seen from Table 2 that the mean deposit due to the settling mist was one-sixth of the mean total deposit. The mist had cleared entirely 1 hr. after the end of spraying and was observed to be not as thick as in some previous similar spraying operations. The short duration and relative thinness of the final mist were probably due to the delay incurred in repairing the compressor.

Results of the treatment

The immediate effect of the spraying was that no moths were seen flying in the stowage by the time the mist had cleared, i.e. the pyrethrum had exerted its knock-down effect.

During the summer of 1945 counts were made at weekly intervals to estimate the numbers of moths present in the stowage. The counts were continued after the spraying, and in Table 3 are shown compared with similar counts in the same stowage during 1943 and 1944.

TABLE 3. *Comparison of estimations of the* Ephestia elutella *moth population in a stowage in a London granary on similar dates in* 1943, 1944 *and* 1945

1943		1944		1945	
Date	Number of moths	Date	Number of moths	Date	Number of moths
10. vi.	1,000	14. vi.	900	12. vi.	1,100
21. vi.	8,200	21. vi.	1,300	21. vi.	6,800
29. vi.	35,800	29. vi.	10,000	28. vi.	69,600
6. vii.	26,400	6. vii.	125,500	5. vii.	173,600
13. vii.	10,500	12. vii.	195,500	12. vii.	149,300
20. vii.	7,800	19. vii.	136,300	19. vii.	68,900
				20. vii.	Stowage sprayed
				21. vii.	1
27. vii.	6,700	25. vii.	72,400	25. vii.	3
3. viii.	2,600	1. viii.	30,800	3. viii.	0

The estimated population of 68,900 moths in the stowage on 19 July was reduced to one living moth when a count was made the day after spraying. Only three living moths were seen subsequently up to 3 August when the weekly counts were discontinued.

Richards & Waloff state that a close estimate of the total number of moths which have emerged in a season, in the conditions of the stowages studied, can be obtained by multiplying the maximum population observed by 1·6. Using this factor it was estimated that the total moth emergence in the stowage for the year was 277,800, but, by the use of another of Richards & Waloff's methods, involving computations based on sex ratio and adult longevities, it was calculated that about 270,300 moths had emerged up to the time of spraying. Since only four moths were seen alive after the spraying, approximately 7500 must have been killed on subsequent emergence by the film of insecticide deposited on exposed surfaces in the stowage.

Visits were made to the stowage on 13 and 25 September to ascertain whether or not the insecticidal treatment had had any effect on the number of migrating *Ephestia* larvae. The estimated numbers were 22,600 on the 13th and 118,100 on the 25th. No further counts were made, as the latter figure and observations in the stowage indicated that there had been no drastic reduction in larval numbers which could be attributed to the spray treatment.

In 1943 the maximum number of larvae (87,500) was found on 9 September, and in 1944 the maximum number (257,900) was found on 14 September. The period between maximum moth emergence and maximum larval migration in 1943 amounted to 72 days, in 1944 to 64 days and in 1945 to 82 days, assuming that 118,100 was the maximum larval count in 1945. Insufficient data have so far been collected to indicate whether the 82-day period

recorded for 1945 implies a delay in reaching the peak of larval migration. If, in fact, this period is longer than normal, it cannot be explained in terms of the physical conditions obtaining in the grain, viz. temperature and moisture content, but may in some way be connected with the application of the spray at the time of moth emergence and oviposition. It will be interesting to make further observations on this point in the future.

Facilities for the observations were arranged by the Ministry of Food, Infestation Branch, whose operators were responsible for the application of the sprays. This investigation was carried out as part of the programme of research of the Pest Infestation Laboratory, and this paper is published by permission of the Department of Scientific and Industrial Research.

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Experiments on the use of ethylene dibromide as a fumigant for grain and seed

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The toxicity of ethylene dibromide to *Calandra oryzae* and *Tribolium* species was determined in the absence and presence of grain. Its toxicity by oral administration was studied on rats and guinea-pigs. The quality of bread baked from flour fumigated with ethylene dibromide was found to be unimpaired. The germination capacity of wheat, barley, maize, peas and beans was not adversely affected by fumigation with ethylene dibromide. The results of large-scale fumigations are reported and the conditions discussed under which ethylene dibromide can be used advantageously as a fumigant for grain and seed stores on a commercial scale. A concentration of 50–75 g./m.^{3} was found satisfactory.

Introduction

In the course of an investigation on the toxicity of over 100 substances, Neifert *et al.* (1925) found that the minimum lethal concentration of ethylene dibromide for *Calandra oryzae*, *C. granaria* and *Tribolium* was 30 g./m.^{3} for an exposure period of 24 hr. in the absence of grain. They recommended further experiments with this substance. The minimum lethal concentrations of over 300 substances for *Calandra oryzae* was determined by Roark *et al.* (1929) using flasks half-filled with grain. For ethylene dibromide they obtained the value of 87 g./m.^{3} for an exposure period of 24 hr. In Palestine, during 1938–9, Plaut *et al.* studied the penetrating power and toxicity of ethylene dibromide, and the encouraging results obtained induced us to undertake a more comprehensive study of the various factors bearing on its practical utilization as a fumigant for grain and seed stores.

Absolute toxicity to insects

(*a*) *Experiments in closed vessels.* The toxic atmosphere was produced in glass vessels of 250–500 c.c. capacity fitted with ground-glass stoppers. Thin-walled ampoules containing a known amount of the fumigant were introduced into the vessels which were then stoppered. The ampoules were then broken by shaking and the stoppered vessels allowed to stand for a period of 24 hr. to ensure complete

evaporation and distribution of the fumigant. The insects were then introduced into the vessel as quickly as possible and kept there for a given time (usually either 4 or 24 hr.). Control experiments have shown that during the introduction of the insects only a small amount of the fumigant escaped from the vessel. In the controls, 10–25 mg. liquid bromine were introduced into glass-stoppered flasks of 250–500 c.c. capacity. After 20–24 hr., when complete evaporation and even distribution was attained, the flasks were opened to the atmosphere for a few seconds and 2 c.c. of 1 % sodium hydroxide solution added to adsorb the bromine. The hypobromite formed was estimated iodometrically and found to correspond to about 95 % of the bromine originally introduced.

(*d*) *Results.* Table 1 gives some typical results on the toxicity of ethylene dibromide to the rice weevil at three different concentrations and periods of exposure. The state of the insects is indicated by: n. (normal = normal behaviour), w. (weak = insects moving with difficulty), v.w. (very weak = insects only moving their legs), and imm. (immobile = no movements at all). It may be seen from this table that concentration and observation. In some cases the period of observation was extended to 10–14 days, but no recovery was observed.

On the other hand, those insects which were still alive after the above-mentioned 48 hr. observation period in a normal atmosphere showed a higher mortality as compared with control insects when kept for a longer period (up to 120 hr.).

TABLE 1. *Toxicity of ethylene dibromide to rice weevil* (Calandra oryzae) *at* 30° C. *Relative humidity about* 50 %

Observation period (hr.) in normal atmosphere	Concentration 2 mg./l. Exposure 24 hr.				Concentration 3·8 mg./l. Exposure 10 hr.				Concentration 7·1 mg./l. Exposure 4 hr.				Control			
	imm. %	v.w. %	w. %	n. %	imm. %	v.w. %	w. %	n. %	imm. %	v.w. %	w. %	n. %	imm. %	v.w. %	w. %	n. %
0	28	44	28	—	4	44	52	—	—	40	60	—	—	—	—	10
23	92	4	4	—	68	4	28	—	68	12	20	—	4	—	—	96
44	100	—	—	—	100	—	—	—	100	—	—	—	16	—	—	84

(*b*) *The choice of the insects.* It was known that the resistance of the same species of insect to a given fumigant might vary considerably, but as the purpose of these experiments was to obtain the data necessary for the use of ethylene dibromide as fumigant under the conditions existing in Palestine, the insects were selected at random from various parts of the country; no attention was paid to the kind of food material on which the insects had been living or to the time of year at which the experiments were carried out. As was to be expected, the results obtained with these insects varied appreciably. Twenty-five to fifty insects were used for each experiment.

(*c*) *The death-point.* Fumigants such as carbon disulphide and hydrocyanic acid have a stupefying effect and insects seemingly dead may recover after some time if put into a normal atmosphere. On the other hand, ethylene dibromide has a delayed action, similar to that of methyl bromide. Insects exposed to ethylene dibromide, even if they seemed normal during the time of exposure, often died 48 hr. later in a normal atmosphere. In all the present experiments, the insects, after having been exposed to the toxic atmosphere, were observed in a normal atmosphere for at least 48 hr. and only then counted as dead or alive. Insects which appeared dead when removed from the toxic atmosphere of ethylene dibromide did not recover during the time of observation. In some cases the period of observation was extended to 10–14 days, but no recovery was observed.

On the other hand, those insects which were still alive after the above-mentioned 48 hr. observation period in a normal atmosphere showed a higher mortality as compared with control insects when kept for a longer period (up to 120 hr.).

(*d*) *Results.* Table 1 gives some typical results on the toxicity of ethylene dibromide to the rice weevil at three different concentrations and periods of exposure. The state of the insects is indicated by: n. (normal = normal behaviour), w. (weak = insects moving with difficulty), v.w. (very weak = insects only moving their legs), and imm. (immobile = no movements at all). It may be seen from this table that concentrations of 2, 3·8 and 7·1 mg./l. for exposure periods of 24, 10 and 4 hr. respectively, are sufficient to cause a 100 % kill. The delayed action of ethylene dibromide is evident in all experiments.

Tables 2 and 3 give comparative data on the toxicity of ethylene dibromide and methyl bromide. The former is even more toxic than methyl bromide which has so far been considered the most toxic fumigant for the rice weevil. The delayed action of ethylene dibromide is more pronounced than that of methyl bromide.

Table 4 compares the minimum lethal concentrations of three common fumigants with those of ethylene dibromide under similar conditions. The concentrations given for ethylene dibromide are the extreme values obtained in about 100 tests. For the three other fumigants only a few tests were carried out, since the results obtained agreed reasonably well with those given by Shepard *et al.* (1937).

Table 5 gives the results obtained in a few tests with *Tribolium* sp. Two interesting facts can be observed from Tables 4 and 5: (1) the very high toxicity of ethylene dibromide compared with that of other fumigants; (2) the toxicity of ethylene dibromide for *Tribolium* is less than that for *Calandra oryzae* in contradistinction to the related compound ethylene dichloride which shows a higher toxicity for *Tribolium* than for *Calandra oryzae* (Shepard *et al.* 1937).

No direct experiments on the toxicity of ethylene dibromide for the other stages of development (eggs, larvae and pupae) of the rice weevil or flour beetle were made. But in many cases, especially in the large-scale fumigations, samples of badly infested grain were removed after fumigation and kept in a warm place for over 2 months. In no case was any reinfestation observed.

guinea-pigs. Nineteen were used, the period of feeding being about 4 months. From Table 6, summarizing some of the results, it may be seen that animals fed with an average daily dose of about 40–50 mg./kg. body weight did not show any abnormal signs during the period of the experiments.

The determination of ethylene dibromide in bread

Table 2. *Toxicity of ethylene dibromide and methyl bromide to* Calandra oryzae. *Exposure* 24 *hr. Temperature* 30° C.

Observation period	$C_2H_4Br_2$ 1·0 mg./l.				CH_3Br 1·06 mg./l.				Control			
	imm.	v.w.	w.	n.	imm.	v.w.	w.	n.	imm.	v.w.	w.	n.
hr.	%	%	%	%	%	%	%	%	%	%	%	%
0	—	—	100	—	20	—	4	76	—	—	—	100
22	24	8	68	—	40	—	—	60	—	—	—	100
48	92	8	—	—	56	—	12	32	4	—	—	96

Table 3. *Toxicity of ethylene dibromide and methyl bromide to* Calandra oryzae. *Exposure* 4 *hr. Temperature* 30° C.

Observation period	$C_2H_4Br_2$ 4·2 mg./l.				CH_3Br 4·1 mg./l.				Control			
	imm.	v.w.	w.	n.	imm.	w.v.	w.	n.	imm.	v.w.	w.	n.
hr.	%	%	%	%	%	%	%	%	%	%	%	%
0	—	—	100	—	—	—	16	84	—	—	—	100
22	32	—	68	—	8	—	8	84	—	—	—	100
48	92	8	—	—	32	—	—	68	—	—	4	96

Table 4. *Minimum concentrations lethal to* Calandra oryzae. *Temperature* 30° C.

Fumigant	4 hr. exposure (mg./l.)	24 hr. exposure (mg./l.)
Carbon disulphide	30– 40	13
Ethylene dichloride	80–150	20–40
Methyl bromide	5– 7	2– 3
Ethylene dibromide	5– 8	1– 2

Table 5. *Minimum concentrations lethal to* Tribolium *sp. Temperature* 30° C.

Fumigant	4 hr. exposure (mg./l.)	24 hr. exposure (mg./l.)
Carbon disulphide	50–70	—
Ethylene dichloride	70–73	25
Methyl bromide	11–13	—
Ethylene dibromide	13–17	2

Toxicity of ethylene dibromide to mammals

The toxicity of ethylene dibromide through inhalation

The vapours of ethylene dibromide, like those of other fumigants, are toxic and the use of a gas mask by the fumigators is essential if they are exposed to such vapours in a closed store.

The toxicity of ethylene dibromide through oral administration

This question was investigated by determining (*a*) the maximum quantity which can be safely administered to animals, and (*b*) the quantity of fumigant retained by bread baked from fumigated flour.

The toxicity of ethylene dibromide by oral administration was determined using rats and

was carried out by combustion over a platinum strip which was heated to 900° C. The bromine formed was estimated by the method of Kolthoff *et al.* (1937) as modified by Brodie & Friedman (1938).

Table 7 summarizes the results obtained. Exps. 1, 2 and 3 were made in order to test the combustion apparatus and the method of analysis. Known solutions of ethylene dibromide in alcohol were burnt. Table 7 shows that the values obtained corresponded fairly well to those expected. In Exps. 4, 5 and 6 samples of 10 g. of ordinary bread were moistened with 2 c.c. of an alcoholic solution of ethylene dibromide of known concentration. The bromine contents of the samples were determined as described above. In Exps. 7, 8 and 9 the values are given which were obtained with samples of 10 g. of bread baked from fumigated flour. Blank analyses carried

out on bread baked from ordinary (unfumigated) flour gave an apparent bromine content of $5 \pm 0{\cdot}5 \times 10^{-6}$ g. bromine per gram sample. The values given in Table 7 are corrected figures obtained by subtracting this value from the experimental results.

These results show that even without previous aeration of the flour, bread baked from fumigated flour will contain less than 2 mg. ethylene dibromide per kg. bread. If it is assumed that 0·5 kg. is consumed daily by a person weighing 50 kg., the consumption will be 0·02 mg. ethylene dibromide per kg. body weight. This figure compared with the amount of ethylene dibromide which was administered to animals without harmful effect, namely, 40 mg./kg., seems to exclude any danger of poisoning by oral administration.

experiment, and samples of the untreated grain were kept for comparison with the fumigated grain at the end of the experiment.

Table 8 gives the results of these tests. It will be seen that even under these very severe conditions (i.e. no desorption of the fumigant, an occurrence seldom encountered in practice) the germinating capacity of the grain is practically unimpaired after 9 months in an ethylene dibromide concentration of 100 g./m.^{3} A concentration of 200 g./m.^{3} had no effect during the first month, but after 3 months the germinating capacity of wheat and barley was slightly impaired.

Table 9 shows the results of similar tests made in actual fumigation experiments. It seems that ethylene dibromide, like ethylene dichloride, has no adverse effect on the germinating capacity.

TABLE 6.* *Toxicity of ethylene dibromide to rats and guinea-pigs*

Animal	Weight (g.) Initial	Weight (g.) Final	Period of feeding (days)	Average daily dose (mg.)	Maximum dose (mg.)	Total dose (mg.)	Method of administering ethylene dibromide
Rat 1	177	220	—	—	—	—	Oil alone (control)
Rat 2	178	220	95	3·4	4·8	320	In oil
Rat 8	163	209	95	4·4	6·8	421	In oil
Rat 12	134	169	77	7·1	10	564	In 50% aqueous alcohol
Guinea pig 1	420	620	94	—	—	—	50% aqueous alcohol alone (control)
Guinea pig 4	280	420	94	15	20	1420	In 50% aqueous alcohol

* One rat which was fed with 20 mg. ethylene dibromide daily died after 3 months. The total dose administered was 1344 mg.

TABLE 7. *Residual ethylene dibromide in bread baked from fumigated flour*

Exp.	Bromine added (mg.)	Bromine found (mg.)
1	0·039	0·038
2	0·039	0·032
3	0·039	0·035
4	0·038	0·030
5	0·046	0·040
6	0·051	0·046
7	—	0·008
8	—	0·007
9	—	0·013

Influence on the germinating capacity

Flasks of 250 c.c. capacity were half-filled with grain. An ampoule containing a known amount of ethylene dibromide was introduced, the flask was stoppered, and the ampoule was broken by shaking. The flasks, which were made completely airtight by sealing the stopper with solid paraffin, were kept at room temperature. The germinating capacity of the grain was examined at the beginning of the

Influence on baking properties

Flour was put in tins of about 2 l. capacity, and 0·4 g. of ethylene dibromide was added. The tins were shaken and then stored for 7 days at room temperature. Bread baked from this flour, with or without 1 hr. aeration before preparing the dough, did not show any abnormality in volume, taste or odour. These results are in accordance with those of Neifert *et al.* (1925).

Factors affecting the use of ethylene dibromide in store rooms

Ethylene dibromide is a liquid with a high boiling-point (131·6° C.), and its vapour pressure at room temperature is therefore low (10 mm. at 22·7° C.), but owing to its high absolute toxicity and high molecular weight compared with other common fumigants, the time needed for the attainment of a certain degree of toxicity in the atmosphere is not long, e.g. it is about one-sixth of that needed for ethylene chloride, although the latter has a vapour pressure about 6 times that of ethylene dibromide.

The distribution of any fumigant in the empty space of the storeroom is mainly to be attributed to convection currents, and in using ethylene dibromide, which is absolutely non-inflammable, electrically driven fans can be safely employed. The penetration of ethylene dibromide into stored products is poor, as it is readily adsorbed by grain. In order to measure this rate of adsorption and compare it with two other common fumigants, the toxicity in the absence and presence of grain was studied in the following way:

A glass vessel of 250–500 c.c. capacity was about half filled with grains of a known moisture content. Ampoules containing the fumigant in amounts larger than those necessary to kill the insects in the absence of grain (see Table 4) were introduced into the vessel and broken. The vessel was then shaken for 24 hr. to produce an even distribution of the fumigant and to facilitate its adsorption by the grains. At the end of this period the insects were quickly introduced into the vessel and kept there for 24 hr. The minimum concentration necessary for a 100% kill was determined and compared with that found for the same time of exposure in the absence of grains. The ratio indicates the relative degree of adsorption of the fumigant by the grains.

Table 10 gives the results obtained in experiments with ethylene dibromide and Table 11 summarizes the results obtained with this and two other fumigants.

The high adsorption of ethylene dibromide explains its poor penetrating ability in comparison with that of methyl bromide, and also accounts for the fact that much larger concentrations must be used in actual fumigation than would be anticipated from the results of the toxicity studies (cf. Tables 4, 5).

TABLE 8. *Influence of ethylene dibromide on the germinating capacity in laboratory tests*

Barley 11·1% moisture; wheat 13·1% moisture. Temperature 20–25° C.				
Duration of exp. (days) ...	0	35	95	280
	%	%	%	%
Wheat control:	97	94	81·5	85
100 mg./l.		92	84·0	84·5
200 mg./l.		98	79	56
Barley control:	98	98	94·5	96·5
100 mg./l.		95	94	86·5
200 mg./l.		91	77·5	80
Barley 10·5% moisture; wheat 10·1% moisture. Temperature 20–25° C.				
Duration of exp. (days) ...	0	38	112	290
	%	%	%	%
Wheat control:	75	80·5	85	85
100 mg./l.		81	78	72
200 mg./l.		79·5	59·5	54·5
Barley control:	100	98·5	98·5	96·5
100 mg./l.		94·5	96·5	76
200 mg./l.		98	87·5	72

TABLE 9. *Influence of ethylene dibromide on the germinating capacity in actual fumigation*

Capacity of store	Kind of container	Kind of commodity	Concentration (g./m.³)	Time of exp. (days)	Germinating capacity: Before fumigation (%)	Germinating capacity: After fumigation (%)
200 l.	Bin	Maize	200	10	90·5	94·5
200 l.	Bin	Peas	200	10	99·5	99·5
200 l.	Bin	Beans	200	10	99	96
60 l.	Wooden cases	Barley	200	9	21	24
60 l.	Wooden cases	Maize	200	9	89	95
60 l.	Metal bins	Maize	200	9	79	79
35 m.³	Concrete	Wheat	110	5	98	99

PRACTICAL FUMIGATION

About 25,000–30,000 tons of grain are produced annually in the Jewish settlements of Palestine, but only about half of this is stored in a way which permits of fumigation. The other half, and the grain produced in Arab villages, is stored in a very primitive manner.

The various modes of storage are as follows: (*a*) Small modern silos of about 200 tons capacity, divided into several chambers and equipped with facilities for the cleaning and the aeration of the grain. (*b*) Storage in bulk: a store which can be made airtight is divided into compartments by means of wooden partitions, and the grain is introduced either manually, or by means of a mechanical elevator. Typical dimensions of a compartment are $4 \times 4 \times 4$ m. The empty space between the compartments is usually occupied by sacks of grain. (*c*) Airtight stores filled to one-third or one-quarter of their capacity with sacks of grain in such a manner that the maximum surface of the sacks is exposed to the atmosphere. (*d*) Small bins of 2–5 tons capacity made from metal sheets.

In the case of silos, the ethylene dibromide was introduced from above during the filling, by sprinkling it over the surface of the grain entering the silo. The fumigant was used in concentrations varying from 50 to 100 g./m.^{3} Although no specially controlled experiments were carried out in silos, ethylene dibromide was used successfully in about fifteen silos having a total capacity of about 3000 tons of grain, no insects being found during the subsequent storage period.

When the grain was kept in sacks, the appropriate amount of ethylene dibromide was sprinkled on their surface and on the walls of the storeroom. A gas-mask was normally used by the fumigator. In a storeroom of 600 m.^{3} the time needed for two men to sprinkle the fumigant was about 5 min. In Table 12 data of controlled experiments are given. The percentage of living insects and their species were determined before fumigation in sacks placed at different points of the storeroom.

With grain in bulk the fumigant was distributed in such a way that the distance between the various points of introduction did not exceed 1 m. This was done by means of an iron pipe ending in a cone which was introduced into the grain at various points and to varying depths. The fumigant was poured into the pipe and in this way the thickness of the layer through which the fumigant had to diffuse was limited to half a metre.

In the cases reported in Table 12, all the insects were found dead when the store was opened after fumigation. Since ethylene dibromide has a delayed action (referred to above) in addition to its immediate effect, it is clear that it would be permissible to reduce either the fumigant concentration or exposure time or both.

TABLE 10. *Toxicity of ethylene dibromide in the presence of wheat*

Glass vessels of 275 c.c. capacity filled with 125 c.c. wheat. 9% moisture. Exposure 24 hr. Temperature 30° C.

Time after exposure (hr.)	Conc. 6·5 mg./l.				Conc. 13·1 mg./l.				Conc. 15·3 mg./l.				Conc. 22·4 mg./l.			
	imm.	v.w.	w.	n.	imm.	v.w.	w.	n.	imm.	v.w.	w.	n.	imm.	v.w.	w.	n.
0	8	—	92	—	8	—	32	60	36	16	16	32	28	12	44	16
24	16	—	84	—	48	12	8	32	64	4	16	16	64	8	16	12
48	48	16	36	—	88	8	4	—	96	4	—	—	100	—	—	—

TABLE 11. *Relative toxicities in the presence and in the absence of wheat*

Fumigant	Ratio
Methyl bromide	2·5 to 3
Ethylene dichloride	8 to 12
Ethylene dibromide	15 to 20

TABLE 12. *Controlled large-scale fumigation with ethylene dibromide*

Store capacity (m.^{3})	Temperature approx. 20° C. Contents	Concentration (g./m.^{3})	Time of exposure (days)
35	20 tons wheat in bags	110	6
140	40 tons wheat and barley in bags	100	6
500	150 tons wheat and barley (about 80 tons in bulk)	75	6
35	About 20 tons wheat and barley in bags	50	6
300	40 tons horsebeans 2 tons atta in bags	75	6
600	170 tons crushed maize in bags	65	5
17	8 tons wheat in bags	88	2

As can be seen, a comparatively long time of exposure was used. (Under conditions prevailing in Palestine, such long exposure periods cause no inconvenience in practice.) The last experiment was carried out in a small storeroom of 17 m.^{3} with the object of determining whether a period of exposure of 5–6 days is really necessary. Other experiments carried out outside Palestine have shown that when a fan is used the exposure period for grain kept in sacks may be reduced to 24 hr. for a concentration of 75 g./m.^{3}

Besides the controlled fumigations, routine fumigations with ethylene dibromide were carried out throughout the country during the years 1943–5. More than 30,000 m.^{3} of storeroom were fumigated during this period. The concentrations used varied from 50 to 100 g./m.^{3}, with 5–10 days of exposure. Reports received from many settlements and from the Field Crop Growers Association indicate that in all cases the use of ethylene dibromide even by inexperienced people completely freed the grain from all infestation. Fumigations were carried out only once a year, and the reports show that no reinfestation occurred. This latter point is important, as it is in all probability the high adsorptivity of the grain for ethylene dibromide which protects it against reinfestation for a considerable time.

Conclusions

From the work carried out so far the following conclusions can be drawn: Ethylene dibromide vapours are highly toxic to *Calandra oryzae* and *Tribolium* sp. Owing to its high toxicity and high specific gravity only a small volume is needed per ton of goods to be fumigated. It can be applied by an inexperienced farmer with a minimum of precautionary measures. It has no effect on the germinating capacity of seeds of wheat, barley, maize, vetch, peas and beans. It affects neither the baking properties of flour nor the taste and odour of the bread. It is, however, very strongly adsorbed and consequently does not penetrate very far into the material being fumigated. Its high retention by commodities may prove to be an advantage by preventing reinfestation. It is absolutely non-inflammable, and electrically driven fans can therefore be used safely within the storeroom to facilitate evaporation, distribution and penetration.

These properties render the use of ethylene dibromide advantageous when the goods to be fumigated do not form a thick layer to be penetrated by the vapours. It is therefore very suitable for fumigating bins and storerooms prior to filling. Experiments have shown that in empty experimental stores of 4 m.^{3} and in a storeroom of 60 m.^{3} a concentration of 15 g. ethylene dibromide per m.^{3} and an exposure of 24 hr. were sufficient for a 100% kill of *Calandra oryzae* and *Tribolium* sp., compared with a concentration of 150 g./m.^{3} required when an ethylene dichloride-carbon tetrachloride mixture is used.

Ethylene dibromide is especially suitable for the fumigation of airtight storerooms filled with sacks, where a layer of only about 20 cm. has to be penetrated, and for fumigation under suitable tents. It is also suitable for the fumigation of grain in large mechanized silos. In this case the fumigant should be introduced continuously with the stream of grain (as is done in the case of chloropicrin).

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The use of toxic polynitro derivatives in pest control

III. The stability of dinitro-*o*-cresol in compounded products

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Under certain conditions dinitro-*o*-cresol (D.N.C.) is easily decomposed to reduction products devoid of ovicidal properties to eggs of apple sucker, winter moth and fruit tree red spider.

Because of this, corrosion of containers in which D.N.C. products are stored may be accompanied by a deterioration of the product.

At the concentration normally used in winter washes (0·1 %), D.N.C. is equally effective whether present as cresol or as the sodium cresylate against eggs of apple sucker and of winter moth, but against eggs of fruit tree red spider the cresol is more ovicidal than the cresylate.

The ease of reduction of D.N.C. (2 : 4-dinitro-6-methyl phenol) raises problems not only in the analytical estimation of the cresol in compounded products (Batt *et al.* 1944) but of the storage stability of such products. First, it must be known whether the products of reduction are of greater or less toxicity than the parent compound. Secondly, since, in the former case, analysis will no longer be a guide to biological efficiency and, in the latter case, a loss of toxicity is involved, it must be known whether reduction will proceed in the compounded product during storage and what precautions are necessary to stop the process.

Biological tests

It has not been found possible to extract from D.N.C.-petroleum oil preparations the products formed by the reduction of the cresol, for reasons which emerged in attempts to prepare the di-amino derivative by direct reduction. The mono-amino cresol only was isolated, and although a crude product thought to be the dihydrochloride was prepared, decomposition with the formation of red dyestuffs followed attempts to separate the amine.

Experience of the susceptibility of the cresol reduction during the analysis of compounded products (Batt *et al.* 1944) indicated that the biological effects of the reaction could be studied merely by the addition of alkali to D.N.C. emulsion containing sulphite lye. It is, however, known that the toxicity of the cresol is influenced by the hydrogen-ion concentration of the spray in which it is applied. Boyce *et al.* (1939) showed that the action, on eggs of *Lygaeus kalmii* Stål, of petroleum oil washes containing dinitro-*o*-*cyclo*hexyl phenol (D.N.O.C.H.P.) decreased as the alkalinity of the wash increased. Barker *et al.* (1945) found that, with D.N.C.-petroleum emulsions against eggs of *Aphis fabae*, about thrice the amount of phenol was required for equal kill at *p*H 9 than at *p*H 4, an effect which with Boyce *et al.* they attributed to the greater toxicity of the phenol when present in the oil phase. Brian (1945), showing that the fungistatic action of D.N.C. was greater in acid than in alkaline media, considered that the effect of *p*H on toxicity can be adequately explained in terms of dissociation of the phenol, the undissociated phenol molecule being more toxic than the phenol anion. In the present work, whatever the explanation of the effect, it is evident that, in comparisons of the toxicity of the cresol in sprays of different *p*H, its results will have to be distinguished from the possible results of the reduction of the cresol.

Preparation of sprays

The mono-amino cresol was prepared as follows: To 5 g. D.N.C. in 40 ml. absolute alcohol, 60 ml. 0·880 ammonia solution was added and a stream of H_2S passed into the solution for 2 hr. The resulting blood-red solution was acidified with HCl, boiled and the precipitated sulphur filtered off. The filtrate was made just alkaline with NaOH, then acid with acetic acid. On standing fine sheaves of crystals separated which were recrystallized from water as long slender red-brown needles, m.p. 173–174° C. Cazeneuve (*C.R. Acad. Sci., Paris*, 1897, **124**, 154) described 2-amino-4-nitro-6-methyl phenol as red-brown needles m.p. 165° (*d*); Hodgson & Ward (*J. chem. Soc.* 1945, p. 665) give m.p. 176°. The reduction of the cresol by tin in HCl solution gave amorphous ill-defined residues from which fine needles, presumably of the dihydrochloride of diamino-*o*-cresol, were recovered. Attempts to liberate the free base lead to decomposition to intensely coloured red-black dyes.

For toxicity comparisons D.N.C. and its mono-amine were dissolved in a mixture of acetone and Carbitol (diethylene glycol monoethyl ether) con-

taining Sulphonated Lorol T.A. (50% triethanolamine dodecyl sulphate). These solutions were diluted with water, immediately before use, to the required strength, and a control was included of the solvents and emulsifiers.

The D.N.C.-petroleum emulsions were prepared either by the dilution of stock emulsions containing the cresol and oil in the proportions required or by mixing equal volumes of diluted emulsions or solutions containing twice the required amounts of cresol and petroleum oil respectively. The stock emulsions contained 66·7% v/v of oil and 33·3% of 10% sulphite lye prepared from syrup of sp.gr. 1·3. Alkali, when needed, was added to the D.N.C. in solution as sodium cresylate prior to the addition of the emulsion and the time interval between mixing and application was noted.

In early tests made primarily to determine the best strength of oil or cresol to use, the two-solution oleic acid method of emulsification (Martin, 1931) was used. The petroleum oil used throughout was Shell JD 2, an oil typical of those used for petroleum winter wash manufacture and of characteristics given under the code P4*a* (Kearns *et al.* 1937). Except for trials in which commercial D.N.C. was used to test the effects of impurities, the cresol recrystallized from alcohol to m.p. 85–86° C. was used, while the sample of 2:4-dinitro-6-*cyclo*hexyl phenol used was of m.p. 105–106° C.

Techniques of bioassay

The eggs of apple sucker (*Psylla mali* Schm.), winter moth (*Cheimatobia* (*Operophtera*) *brumata L.*) and fruit tree red spider mite (*Oligonychus ulmi* Koch.) were used in the bioassays. With each species a technique was developed so that the eggs were laid under controlled conditions that assured maximum viability and accuracy of examination.

The sucker eggs were laid by suckers caged on selected clean apple shoots, the infested twigs not being removed from the tree until just before treatment. The eggs of winter moth were laid on cut lengths of well-spurred wood placed in glass-tube cages; as soon as oviposition was completed, the twigs were removed and kept outdoors, one end embedded in damp sand to prevent drying out. The red spider eggs were laid on clean damson trees and the infested twigs cut when required for treatment.

Just before treatment the egg-bearing shoots were collected and doubtful material discarded. Twigs, selected at random, were set aside so that each treatment had 100–200 eggs of winter moth, 200–300 of sucker and 2000–10,000 of red spider.

All treatments were applied by dipping the twigs into the freshly prepared wash for 1 sec., removing and immediately re-dipping for 2 sec. to ensure even wetting. In the case of the alkali additions to sulphite lye emulsions, the time between mixing and dipping was never more than 15 min. The twigs were then mounted vertically on cabinet pins to permit uniform and rapid drying. They were removed outdoors and placed in damp sand protected in wire-netting cages from birds and mice, but subjected to normal weathering.

Just before egg hatch, the twigs were removed to the laboratory and those with sucker and winter moth eggs were cut to convenient lengths and placed into tubes. In the case of red spider lengths or segments of twig bearing 500–1500 eggs were saved. The tubes were placed vertically in racks, the inside of the top of each tube being lightly smeared with banding grease so that, as the tubes were exposed to light, the insects or mites which hatched were caught on the grease where they were subsequently counted. The total eggs were also counted.

Results

The results are assembled in Tables 1–3, which include date of treatment, the total number and percentage hatch of eggs. As each total comprised counts from the separate twigs used for each treatment an estimate of the uniformity of response of the various samples per treatment was possible. This estimate is given as the figure for (χ^2), and although a marked heterogeneity is displayed in the untreated eggs the pertinent differences in hatch are sufficiently large to render further statistical treatment unnecessary.

Discussion

The reduction of one nitro group of D.N.C. to give 2-amino-4-nitro-6-methyl phenol has suppressed ovicidal properties towards eggs of winter moth (Exp. 28), apple sucker (Exp. 65) and red spider (Exp. 174). A similar loss of toxicity has been found in contact insecticidal and stomach poison properties (Martin *et al.* 1944). It might reasonably be suggested that the toxicity of the parent nitro cresol is associated with *in vivo* reduction, leading either to a fatal disturbance of the oxidation-reduction mechanism of the egg or to toxic reduction products. The present results indicate that the reduction products are non-toxic or cannot penetrate into the egg.

The toxicity of products of reduction beyond the mono-amino stage can be directly assessed only from those trials in which the cresol was exposed to alkaline reduction. The complication that the toxicity of the cresol itself is dependent on phase distribution or on degree of dissociation has already been mentioned and to this is added the fact that the concomitant petroleum oil is ovicidal, at least to eggs of winter moth and red spider. The simplest case is therefore ovicidal action on eggs of apple sucker on which the petroleum oil has little effect even at 5% (Table 2, Exp. 39). Moreover, the toxicity of D.N.C. is little affected either by dissociation of the cresol

(cf. Exps. 61 and 62) or by the distribution of the cresol between oil and aqueous phases (cf. Exps. 42 and 47). The addition of alkali has, however, caused a drastic loss of toxicity (cf. Exps. 47 and 48, 49 and 50, and 51), which is attributable to alkaline reduction by the reducing sugars present in the spray.

Similar tests of D.N.C.-petroleum sulphite lye sprays with and without added alkali on winter moth eggs revealed only the susceptibility of these eggs to petroleum oils, but it is apparent from the results given in Table 1 that the ovicidal action of the cresol is little influenced by dissociation. In parallel tests of the cresol alongside its sodium salt (Exps. 24 and 25, and 199 and 200) the concentration may have been too high to show differences, but in the 1938 tests the cresylate (≡0·1 % D.N.C.) showed high toxicity (Exps. 601, 602 and 604), whilst in the 1939 tests the soap emulsions in which the cresol is present as cresylate was as effective as sulphite lye emulsions containing the cresol. The corresponding *cyclo*hexyl

TABLE 1. *Tests on eggs of winter moth*

Exp. no.	Treatments: Active ingredients	Treatments: Auxiliary materials	Total eggs	% hatch	No. of samples	χ^2
Dipped 14. iii. 38						
601	0·1 % D.N.C. (Na salt)	0·6 % oleic acid + 0·1 % NaOH	505	14·3	4	14·8
602	0·2 % D.N.C. (Na salt)		236	4·7	3	6·1
604	0·1 % D.N.C. (comm.) Na salt		362	5·5	3	32·2
606	0·1 % D.N.C. + 3 % JD_2		566	2·8	4	7·0
612	0·1 % D.N.C. (comm.) + 3 % JD_2		380	1·6	2	0·9
566	Nil		1082	84·8	5	84·7
Dipped 15. iii. 39						
661	0·1 % D.N.C. + 2 % JD_2	0·6 % oleic acid + 0·1 % NaOH	59	5·1	5	2·3
662	0·1 % D.N.O.C.H.P. + 2 % JD_2		58	0·0	7	—
660	Nil	—	232	85·8	9	19·5
665	0·1 % D.N.C. + 2 % JD_2	0·1 % sulphite lye	129	1·6	6	3·4
666	0·1 % D.N.O.C.H.P. + 2 % JD_2		94	0·0	3	—
Dipped 5. i. 45						
24	0·225 % D.N.C. (cresol)	1·0 % acetone + 1·4 % Carbitol + 0·14 % S. Lorol T.A.	131	0·0	5	—
25	0·225 % D.N.C. (Na salt)		161	0·0	5	—
28	Amino-nitro cresol (≡0·225 % D.N.C.)		206	91·7	5	2·77
27	Nil		133	62·4	5	41·92
Dipped 13. iii. 45						
199	0·225 D.N.C. (cresol)		249	1·2	5	10·77
200	0·225 D.N.C. (Na salt)		279	0·4	5	3·17
202	Nil		141	90·8	5	4·30

TABLE 2. *Tests on eggs of apple sucker*

Exp. no.	Treatments: Active ingredients	Treatments: Auxiliary materials	Total eggs	% hatch	No. of samples	χ^2
Dipped 8. ii. 45						
39	5 % JD_2	0·25 % sulphite lye	311	65·0	5	8·32
42	5 % JD_2 + 0·1 % D.N.C. (cresol)	Do.	230	4·8	4	17·27
47	5 % JD_2 + 0·1 % D.N.C. (Na salt)	Do.	254	9·4	4	13·60
48	5 % JD_2 + 0·1 % D.N.C. (Na salt)	0·25 % sulphite lye + *N*/20 NaOH	170	58·8	5	11·23
49	5 % JD_2 + 0·225 % D.N.C. (Na salt)	0·25 % sulphite lye	281	10·0	4	11·87
50	5 % JD_2 + 0·225 % D.N.C. (Na salt)	0·25 % sulphite lye + *N*/20 NaOH	211	70·1	6	4·78
51	5 % JD_2 + 0·337 % D.N.C. (Na salt)	Do.	346	71·8	6	45·07
67	Nil	0·25 % sulphite lye	567	76·7	4	15·72
68	Nil	0·25 % sulphite lye + *N*/20 NaOH	253	85·8	6	20·06
61	0·225 % D.N.C. (cresol)	1·0 % acetone + 1·4 % Carbitol + 0·14 % S. Lorol T.A.	189	2·1	4	1·24
62	0·225 % D.N.C. (Na salt)		146	7·5	5	6·11
65	Amino-nitro cresol (≡0·225 % D.N.C.)		203	89·2	4	1·26
64	Nil		241	86·7	4	5·88

phenol at 0·1% in 2% JD_2 inhibited egg hatch whether present as phenol or phenate (Exps. 662, 666).

In the tests on red spider eggs a demonstration of the effects of alkaline reduction was frustrated by the low toxicity of the cresol in alkaline sprays. The failure of the cresylate in field trials against *O. ulmi* difference, the ion being non-ovicidal. In these tests, in which oil was also present (Exps. 625, 279, 280 and 285), toxicity is attributable to the oil itself.

The consistent non-toxicity of the amino-nitro-cresol and the loss of ovicidal properties to apple sucker of D.N.C. under conditions permitting alkaline reduction provide the answer to the first

Table 3. *Tests on eggs of red spider*

Exp. no.	Treatments: Active ingredients	Treatments: Auxiliary materials	Total eggs	% hatch	No. of samples	χ^2
Dipped 16. iii. 38						
623	0·1% D.N.C. (Na salt)	0·6% oleic acid + 0·1% NaOH	1385	88·5	4	1·10
624	0·2% D.N.C. (Na salt)	Do.	694	63·8	5	27·38
625	0·1% D.N.C. (Na salt) + 3% JD_2	Do.	1339	11·9	5	13·18
626	Nil	Do.	2313	96·9	5	13·27
Dipped 13. ii. 45						
174	Amino-nitro cresol (≡0·225% D.N.C.)	1·0% acetone, 1·4% Carbitol + 0·14% S. Lorol T.A.	1394	94·5	10	4·22
161	Nil	Do.	1817	93·2	10	14·34
Dipped 28. iii. 45						
285	0·1% D.N.C. + 3% JD_2	0·25% sulphite lye	1153	1·6	5	19·71
279	0·1% D.N.C. (Na salt) + 3% JD_2	Do.	562	0·2	5	5·32
286	0·1% D.N.C. (Na salt) + 3% JD_2	0·25% sulphite lye + $N/20$ NaOH	983	1·0	5	17·45
284	Nil	0·25% sulphite lye + $N/20$ NaOH	671	88·2	5	4·08
294	0·225% D.N.C.	1·0% acetone, 1·4% Carbitol + 0·14% S. Lorol T.A.	823	6·4	5	40·49
296	0·225% D.N.C. (Na salt)	Do.	850	88·0	5	8·31
295	Nil	Do.	547	89·4	5	3·51

Table 4. *Storage tests of D.N.C. petroleum emulsions*

Metal strip	Sulphite lye emulsion % by wt. D.N.C. after $4\frac{1}{2}$ months	Sulphite lye emulsion % by wt. D.N.C. after $9\frac{1}{2}$ months	Casein ammonia emulsion % by wt. D.N.C. after 8 months	Casein ammonia emulsion % by wt. D.N.C. after 12 months
No strip	2·11	2·10	1·44	—
	2·13	2·09	1·44	—
Unpolished steel	0·35	—	1·42	—
	0·38	0·07	1·39	—
Polished steel	0·38	0·40	1·50	—
	—	0·44	1·46	—
Tinned steel	1·70	1·25	1·42	—
	—	1·27	1·43	—
Zinced steel	1·78	1·73	—	1·50
	1·78	1·73	—	1·51
Resin-coated steel	—	1·42	—	1·60
	—	1·45	—	1·60

has previously been recorded (Kearns & Martin, 1939) and is confirmed in the laboratory tests (Table 3, Exps. 623, 624), while in the direct comparison at the high concentration of 0·225% (Exps. 294, 296) the cresylate proved non-ovicidal whereas the cresol permitted only 6·4% hatch. Since in the latter experiments there was no oil phase present, it would appear that dissociation is the explanation of the question raised in the introduction: the products of reduction of D.N.C. have insignificant ovicidal properties. The chemical estimation of the cresol content surviving reduction in compounded products will therefore give a reliable assessment of ovicidal properties and can be used for the examination of the second question: Under what conditions will reduction proceed in compounded products?

Chemical tests

In general the danger of the use of emulsifiers and other surface active supplements with reducing properties in compounding D.N.C. products is self-evident and is illustrated by the addition of alkali to sulphite lye emulsions in the above recorded experiments. A less obvious possibility is electrolytic reduction due to corrosion of the mild steel drums in which D.N.C.-petroleum emulsions are usually packed.

As typical examples of D.N.C.-petroleum emulsions, one was chosen of acid *p*H, a sulphite lye emulsion made by the method described by Kearns & Martin (1941), known to be corrosive; the second was a proprietary product with casein-alkali as emulsifier and with excess of ammonia added to delay corrosion at the air-metal-emulsion boundary. Aliquots of the emulsions were placed in 4 oz. bakelite stoppered bottles with and without metal strips cut from the usual types of container used for packing D.N.C. emulsions. The strips were of mild steel, polished and unpolished, of tinned steel, of galvanized iron and of steel coated with a protective synthetic resin. The cut edges of the strips (3 × 1 in.) were coated with a bitumen paint and the bottles were stored in the dark at 25° C. for periods up to 1 year, when the contents were analysed (Batt *et al.* 1944). The results are included in Table 4.

Discussion

Loss of D.N.C. on storage occurred in all cases in which strips of metal were included in the sulphite lye emulsion. Inspection of the strips showed, however, that the bitumen protection of the cut edges of the strips had become softened and detached, while the tinned, zinced and resin coated strips showed slight corrosion only at the exposed edge. Had the protective coatings been complete, the loss of D.N.C. from these treatments would have been less. In the polished and unpolished steel treatments, the sulphite lye emulsion deepened in colour until it was almost black and in the analysis much dark-coloured insoluble sludge appeared in the separating funnels. The casein ammonia emulsions retained their deep orange colour throughout, no corrosion of the metal strips was seen and no loss of D.N.C. was detected after storage for 12 months.

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Studies upon the copper fungicides. IX.

Investigations with the exudate from fungus spores

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Determinations have been made of the copper dissolved from pure copper compounds by water, by a standardized solution of the exudate of *Neurospora sitophila* spores, by standard solutions of malic and succinic acids and their sodium salts, and by glycine and mannitol.

The results compared with the known fungicidal performances of the copper compounds show that the amounts of copper yielded to water and to the standardized spore extract or glycine solution provide an indication of fungicidal properties.

Although soluble copper in excess of that dissolved by water can only appear from alkaline Bordeaux deposit by complex ion formation, results show that this is not necessarily the only means by which copper can dissolve from other copper compounds.

In a chemical investigation of the exudate of *N. sitophila* spores, mannitol, succinate and fumarate have been isolated. None of these materials can dissolve an appreciable amount of copper from alkaline Bordeaux deposit. Malate, found by other workers to be a constituent of the exudate of *N. sitophila* spores cultured on potato dextrose agar, has not been detected in such a solution from spores grown on malt agar. The quantity of mannitol in spore exudate has been shown to depend on the nutrient medium on which the fungus is grown.

Introduction

The fact that soluble copper could be produced from dried Bordeaux mixture deposit by the solvent action of the exudate or lysate from fungus spores was demonstrated experimentally by McCallan (1930) and McCallan & Wilcoxon (1936). In Part VI of this series (Wain & Wilkinson, 1943), further supporting evidence was recorded and, on the basis of these results, a hypothesis on the mechanism of the fungicidal action of copper was put forward.

That not all compounds of copper are fungicidal is well known. Marsh (1938), for example, reported the absence of fungicidal properties in cuprous thiocyanate, cupric ferrocyanide and copper phthalocyanine whilst Martin *et al.* (1942), in their critical examination of some forty pure copper compounds, found wide differences in fungicidal activity. The question therefore arises, if toxicity to the fungus spore is due to the liberation of soluble copper under the influence of spore exudate, whether poor fungicidal performance is due to non-solubility in this solvent, for if this is the case a chemical method for assessing the fungicidal value of insoluble copper compounds becomes feasible.

In the present exploratory work, for which the compounds used by Martin *et al.* (1942) were available, determination of soluble copper produced from some of these materials under the influence of a standard solution of spore exudate were made. Such a solution, however, would be somewhat unsatisfactory for routine use as a solvent owing to the difficulty of standardization. Further, these solutions, when allowed to stand, rapidly become contaminated with bacteria with a loss of their capacity to dissolve copper (Wain & Wilkinson, 1943), so that it is necessary to maintain sterile conditions until immediately before use. The use of a solution of spore exudates might be avoided if the active substances present in the exudate were known, for then the solubility effect could be obtained using a solution of one or more of these active materials.

The solubility data already presented (Wain & Wilkinson, 1943) showed that solutions of many types of organic substance are active in dissolving copper, the mechanism being one of complex ion formation, but of these only a limited number are likely to occur in the exudate from fungus spores. McCallan & Wilcoxon, working with the exudate of the spores of *N. sitophila*, found malate and indicated the presence of amino acids—all substances capable of dissolving copper from Bordeaux mixture deposit.

Experimental

Materials and methods

Copper compounds

Compounds of known fungicidal performance were chosen from those listed in Part V of this series (Martin *et al.* 1942). In order to obtain comparable results the substances were weighed out and triturated under distilled water to provide suspensions containing 0·1 % copper.

Spraying

The suspensions were sprayed on to the interior of 14 cm. Petri dishes for a timed interval using

* Now at Wye College (University of London), Kent.

Evans & Martin's (1935) atomizer. The plates were allowed to dry completely (for at least 4 hr.) before use.

Solvents

(*a*) *Standard solution of spore exudate. N. sitophila* was grown on 2% malt agar at 25° C., the spores collected after 7 days and the spore filtrates obtained as already described (Wain & Wilkinson, 1943). 25 ml. lots of a solution containing the exudate from 124×10^9 spores in 1000 ml. glass-distilled water were pipetted into 100 ml. conical flasks and 25 ml. glass-distilled water was added to each. The flasks and contents were then steam sterilized. When required for a shaking experiment, the contents of one flask were made up to 310 ml. with glass-distilled water and 50 ml. of this solution, equivalent to the exudate from 50×10^7 spores, was used per dish.

(*b*) *Solutions of pure chemical substances.* These were made up at a concentration of *N*/1000 or *M*/1000 with glass-distilled water as described previously (Wain & Wilkinson, 1943).

Shaking

This was carried out for 17 hr. as described previously (Wain & Wilkinson, 1943).

Analysis

After shaking, the liquid contents of each dish were centrifuged. In some cases it was necessary to centrifuge up to 40 min. at 2500–3000 r.p.m. in order to obtain clear solutions for analysis. Copper determinations were made on the clear centrifuged solutions by the method already described (Wain & Wilkinson, 1943).

Spore filtrates for chemical examination

Suspensions of *N. sitophila* spores grown on 5% malt agar and on a bread mulch agar containing 15% moist bread were allowed to stand for 16 hr. at 14° C. The spores were then filtered off and the filtrates after Seitz filtration and sterilization at 10 lb./sq.in. for 30 min. were accumulated until sufficient was available for analysis. It had been already shown (Wain & Wilkinson, 1943) that steam sterilization does not affect the capacity of these solutions to dissolve copper from 4:4:50 Bordeaux mixture deposit.

Results

Copper dissolved by water and by the standard solution of spore exudate

The results obtained using twenty-two substances are assembled in Table 1. Also included in the Table is an indication of fungicidal performance based on the results published in Part V of this series (Martin *et al.* 1942).

Copper dissolved by solutions of pure chemical substances

The solubility figures obtained using these solvents against seven selected copper compounds are given in Table 2. For comparison, the glass-distilled water and spore exudate figures are also given.

Chemical investigations on spore filtrates

(*a*) *Filtrates from spores grown on 5% malt agar*

A sterilized solution in which $877{,}590 \times 10^6$ spores had been suspended was available. A portion of this diluted so that 1 ml. was equivalent to 100×10^6 spores had *p*H 5·6, sp.gr. 1·0003 at 60° F., and contained 1·505 mg. soluble solids and 0·025 mg. total N per ml. Potassium, phosphate and traces of sodium and calcium were also present.

The bulk of the solution was concentrated at 40° C. under reduced pressure to about 2 l. and treated with 20 ml. of a cold saturated solution of neutral lead acetate. After standing overnight the precipitated lead salts were centrifuged and washed on the centrifuge with small quantities of water.

Treatment of lead salts. The material was suspended in water, decomposed with hydrogen sulphide and the insoluble lead sulphide removed. The filtrate, which contained free organic acids, gave a blue coloration with triketohydrindene hydrate ('Ninhydrin reagent') indicating the presence of α-amino acids. Qualitative tests for citrate malonate and pyruvate and the specific test for malate described by Challenger & Klein (1929) were negative.

A portion of the solution was neutralized with sodium carbonate and refluxed for 2 hr. with an equivalent amount of *p*-phenylphenacyl bromide after addition of alcohol. The solid product, recrystallized thrice from benzene, yielded a substance, m.p. 206–208° C., which did not depress the melting point of the di-*p*-phenylphenacyl ester of succinic acid, m.p. 209–210° C. The acids from the main bulk of the solution were esterified and distilled under a pressure of 12 mm. No crystalline hydrazide was obtained on treating the distillate with a solution of hydrazine hydrate (compare McCallan & Wilcoxon, 1936).

Treatment of main solution after removal of lead salts. The excess lead was precipitated with hydrogen sulphide and the filtrate, after removing lead sulphide, was evaporated down under reduced pressure to a sticky oil. This was dissolved in the minimum quantity of boiling 80% alcohol. On standing, a crystalline solid separated out which was shown by qualitative tests and mixed melting point to be mannitol. Recrystallized from methyl alcohol it appeared as long white silky needles, m.p. 166–167° C. (Found: C, 39·63; H, 7·85%. Calc. for $C_6H_{14}O_6$: C, 39·54; H, 7·74%.) Identity was further confirmed by

preparation of the acetyl derivative, m.p. 121–123° C., a melting-point not depressed by admixture with an authentic specimen of hexa-acetyl mannitol, m.p. 123° C.

The yield of crude mannitol, m.p. 163–166° C., was 2·411 g., representing 0·2940 mg./100 × 10^6 spores.

for several days in a vacuum desiccator partially solidified. The solid portion was filtered and recrystallized thrice from about 0·5 ml. quantities of water. The crystalline product, which decolorized acidified permanganate but not bromine water, partially sublimed on heating and gave an indefinite melting-point. In a sealed tube, however, the

TABLE 1. *Copper dissolved by water and by standard solution of spore exudate*

Compound	Copper dissolved by: Glass-distilled water p.p.m. (av. of duplicates)	Copper dissolved by: Solution of spore exudate (1 ml. = 10 × 10^6 spores) p.p.m. (av. of duplicates)	Difference p.p.m.	Fungicidal properties
Cupric oxide	0·14	0·49	0·35	Poor
Basic copper arsenate	0·19	0·50	0·31	Poor
Cuprous thiocyanate	0·31	0·51	0·20	Poor
Cupric sulphide	0·34	0·35	0·01	Fair
4:4:50 Bordeaux	0·40	1·52	1·12	Excellent
Copper oxychloride	0·57	1·64	1·17	Good
Cupric phosphate	0·51	3·83	3·32	Excellent
Cuprous cyanide	0·89	0·93	0·04	Poor
4:6:100 Burgundy	0·96	2·35	1·39	Excellent
Basic copper carbonate	1·00	2·21	1·21	Excellent
Copper mucate	1·85	2·17	0·32	Good
Copper oxysulphate	1·29	3·97	1·68	Good
4:5:100 Burgundy	1·27	3·02	1·75	Excellent
Cuprous oxide (red)	1·52	2·47	0·95	Fair
Copper cystinate	1·84	1·89	0·05	Poor
Cuprous oxide (yellow)	3·14	4·68	1·54	Excellent
Copper sebacate	4·78	6·88	2·10	Excellent
Copper oxalate	6·05	8·05	2·00	Good
Copper aspartate	17·09	20·34	3·25	Good
Copper β-phenylalaninate	23·92	28·61	4·69	Fair
Copper benzoate	27·51	28·78	1·27	Excellent
Copper tyrosinate	29·43	31·70	2·27	Fair

TABLE 2. *Solubility of various copper compounds in solutions of pure chemical substances*

Copper compound	p.p.m. copper dissolved by: Glass-distilled water	Solution of spore exudate (1 ml. = 10 × 10^6 spores)	*N*/1000 *l*-malic acid	*N*/1000 Na-*l*-malate	*N*/1000 succinic acid	*N*/1000 Na succinate	*N*/1000 glycine	*M*/1000 mannitol	Fungicidal properties
Basic copper arsenate	0·19	0·50	17·9	0·38	10·7	0·14	2·03	0·14	Poor
Cuprous thiocyanate	0·31	0·51	1·03	—	2·31	0·90	3·50	0·52	Poor
4:4:50 Bordeaux	0·40	1·52	15·65	13·0	0·63	0·62	27·06	—	Excellent
Copper oxychloride	0·57	1·64	43·4	4·52	34·6	0·75	28·9	0·38	Good
Cupric phosphate	0·51	3·83	41·1	8·36	32·2	0·66	27·1	0·45	Excellent
4:5:100 Burgundy	1·27	3·02	35·8	21·0	—	—	32·51	—	Excellent
Copper mucate	1·85	2·17	3·83	—	40·1	2·78	5·29	2·40	Good

The mother liquors after removal of mannitol were evaporated under reduced pressure. The residue was then dissolved in about 25 ml. water, the solution brought to *p*H 1 with phosphoric acid and continuously ether extracted for 7 days. On removing the ether a brown oil was obtained which on standing substance melted at 280–283° C. (uncorr.) and a mixed melting-point with fumaric acid (m.p. 286° C. (sealed tube)) gave no depression. That the substance was fumaric acid was confirmed by preparation of the *p*-phenylphenacyl ester, m.p. 233–236° C., a melting-point not depressed by admixture with the

di-*p*-phenylphenacyl ester of fumaric acid, m.p. 237–239° C. The mother liquors from the three water crystallizations were allowed to evaporate in a vacuum desiccator and the oily solid so obtained was crystallized twice from a mixture of chloroform and alcohol. The product, m.p. 186·5–188° C., gave the qualitative tests for and did not depress the melting-point of succinic acid, m.p. 186–187° C. The *p*-phenylphenacyl ester (m.p. 208–210° C.) was also prepared and again a mixed melting-point with an authentic specimen, m.p. 209–210° C., showed no depression.

The oily filtrate obtained after removing fumaric and succinic acids gave qualitative tests for lactic acid but was not examined further.

(*b*) *Filtrates from spores grown on bread mulch agar*

A filtrate in which $552{,}000 \times 10^6$ spores had been suspended was examined for mannitol as described above. 2·462 g., m.p. 162·5–166° C., were thus obtained, representing 0·4461 mg. mannitol per 100×10^6 spores.

Discussion

(*a*) *Solubility in the standard solution of spore exudate*

Since no attempt has been made to show that a final solution equilibrium was reached between the solvent and the deposit, the figures in Tables 1 and 2 must be regarded as relative only. In the case of Bordeaux mixture, however, such equilibrium has been shown to be reached after 12 hr. shaking.

The figures in Table 1 indicate that, for any particular compound, two factors contributing to its success or failure as a fungicide might be involved. The first is its water solubility. Thus, for example, a compound so soluble as copper benzoate might be expected to prove fungicidal whether spore excretions are involved or not. There is evidence, however, that the form in which the compound yields copper to water is important. Thus Martin *et al.* (1942) indicated that in copper sebacate, the molecule and in cupric cuprimalate, the complex ion might be the agents actually toxic to the spore. Again, in spite of their relatively high water solubility, the copper salts of β-phenylalanine and tyrosine are not good fungicides, the soluble copper in these cases occurring as large complex ions which probably lack the ability to penetrate the spore wall. Further, these ions would persist as such in solution if they were more stable than those which the substances present in spore excretions could form with copper (Wain & Wilkinson, 1943).

The second factor contributing towards fungicidal performance might be associated with the amount of copper dissolved in excess of the glass-distilled water figure by the standard solution of spore exudate.

Referring to Table 1, basic copper arsenate, cuprous thiocyanate and cupric sulphide* for example, which are not appreciably dissolved by water or by spore exudate, are not good fungicides, whereas 4:4:50 Bordeaux mixture, cupric phosphate, 4:5:100 and 4:6:100 Burgundy mixtures, copper sebacate and oxalate, which yield considerable quantities of copper to spore exudate, show a good fungicidal performance. With other compounds such as copper oxychloride and copper mucate, the correlation between solubility figures and fungicidal properties is not close.

(*b*) *Solubility in solutions of pure chemical substances*

The solvents used in this preliminary investigation, the results of which are given in Table 2, were selected for specific reasons. As already stated, McCallan & Wilcoxon (1936) isolated malate from the exudate of *N. sitophila* spores and gave evidence of the occurrence of amino acids. Mannitol, succinate and fumarate have been isolated from such a solution by the writers. An indication of the capacity of these and other compounds to dissolve copper from alkaline Bordeaux deposit has already been given (Wain & Wilkinson, 1943). Here it was shown that malic acid, malate and glycine dissolved copper readily by complex ion formation, but succinic and fumaric acids and their sodium salts dissolved only small amounts, an effect due to the difficulty in the formation of a seven-membered chelated cation. Mannitol, unable to form a soluble complex with copper was inactive with Bordeaux. These earlier results are substantially confirmed by those given in Table 2.

But whereas copper can only dissolve from an excess of alkaline Bordeaux deposit by complex formation so that certain acids and their salts can be equally active in dissolving copper, with other copper compounds this does not appear to be the case. Thus, basic copper arsenate and copper oxychloride are both dissolved more readily by malic and by succinic acids than by the salts of these acids. This is presumably the simple solution of basic derivative in acid with the formation of cupric ions, an effect not possible with alkaline Bordeaux owing to the presence of calcium carbonate in the deposit (Wain & Wilkinson, 1943). Cupric phosphate and mucate are also dissolved by succinic acid, but not by sodium succinate.

Of the solvents used, glycine appears to dissolve appreciable amounts of copper from compounds known to possess good fungicidal properties (e.g. Bordeaux, cupric phosphate, Burgundy mixture),

* The case of cupric sulphide is complicated by its slow oxidation on exposure to moist air to cupric sulphate.

yet only relatively small quantities from basic copper arsenate and cuprous thiocyanate, both poor fungicides. The solubility effect of glycine on copper mucate is also similar to that of the standard solution of spore exudate.

Of the solvents studied then in this preliminary work, $N/1000$ glycine appears to give results closest to those given by spore exudate and with the limited number of materials studied, the solubility in glycine would appear to give an indication of fungicidal performance. It may be noted here that Horsfall *et al.* (1937) found that the solubility of different samples of copper oxide in $N/10$ glycine gave a useful indication of their fungicidal values.

(c) Chemical investigations on spore filtrates

McCallan & Wilcoxon's (1936) indication that amino acids are present in the exudate of *N. sitophila* spores is supported by the results obtained in the present work. The writers, however, were unable to detect malate, which according to McCallan & Wilcoxon formed 3·1 % (estimated as malic acid) of the soluble solids exuded. These workers also isolated malate from their material in the form of its crystalline hydrazide. It must be emphasized here, however, that in the present work the *N. sitophila* spores were cultured on malt agar, whereas potato dextrose agar was used by McCallan & Wilcoxon. The only other notable difference in the two investigations was that the writers examined extracts which had been steam sterilized, whereas McCallan & Wilcoxon used unsterilized solutions some of which presumably had been allowed to stand for several days. In Part VI of this series it was shown that, whereas steam sterilization does not affect the capacity of these solutions to dissolve copper, this capacity was greatly affected by bacterial contamination.

The sensitive and specific test for malate described by Challenger & Klein (1929) was negative, using a highly concentrated solution of our fresh spore exudate.

The presence of three other compounds in the material exuded from *N. sitophila* spores has been demonstrated by the writers, namely mannitol, succinate and fumarate. None of these is appreciably active in dissolving copper from dried Bordeaux deposit, although as already pointed out succinic and fumaric acids might readily dissolve copper from other copper compounds.

The amount of crystalline mannitol obtained from the exudate of *N. sitophila* spores grown on 5 % malt agar, 0·29 mg./100×10^6 spores, represented almost 20 % of the total solids exuded. The weight isolated from the spores grown on bread mulch agar was 0·45 mg./100×10^6 spores. Although these figures ignore the small amounts of mannitol still present in the 80 % alcohol mother liquors, they do suggest that the quantity of a particular constituent exuded by spores might depend on the medium on which they were grown. This is of interest in connexion with results already presented in Part VI of this series, for here the total quantities of soluble solids produced by spores were shown to depend on the culture medium. It also indicates that the detection of malate in spore extracts by McCallan & Wilcoxon and the failure to do so by the writers can be attributed to differences in the nutrient media.

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Field trials of copper fungicides for the control of potato blight

III. Low-copper fungicides

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(With 1 Text-figure)

In trials at Beaford, north Devon, and at Galmpton, south Devon, in 1945, proprietary preparations of copper oxychloride, cuprous oxide, and cuprammonium, used at low-copper dosage (0·063 % Cu), were compared with Bordeaux mixture at the same and at higher dosages for the control of potato blight under two commonly recurring conditions of disease incidence, respectively early and late, which were closely defined by foliage decay curves.

All the copper fungicides were tested with and without added adhesive of caseinate-rosin soap type, and two *sulphur* fungicides: lime sulphur (1 in 50) and Dithane 14 (1 in 200) with zinc sulphate and lime, were also included.

The trials were made on four-times replicated plots at both centres; special precautions were taken to avoid confusion of the observations by concurrent mineral deficiency and virus disorders; and the treatments were compared by three criteria: (1) direct foliage protection, (2) spray retention and (3) indirect effect on yield. The number of spray applications was reduced to a minimum to avoid masking the superiority of the better treatments, and timed as nearly as possible for maximum effect in relation to weather conditions. Thus two applications were given at Galmpton and three at Beaford.

None of the proprietary copper fungicides used at low-copper dosage, with or without added adhesive, equalled plain (4.5.40) Bordeaux mixture in spray retention or direct protective effect; and none gave any significantly better protection than Bordeaux mixture at correspondingly reduced copper dosage (1.1½.40).

With the few well-timed and thorough sprayings given, however, the ¼% Bordeaux mixture itself gave almost sufficient protection for the production of a full crop, and the trials indicated horticultural conditions under which low-copper fungicides can be used with success, while pointing to the important 'factor of safety' secured by using 1 % Bordeaux mixture in routine agricultural practice.

Both of the sulphur fungicides were less effective than ¼% Bordeaux mixture.

Introduction

A number of proprietary copper fungicides now on the market have advantages over Bordeaux mixture for potato spraying in that they simplify the preparation of the spray fluid. Most of these materials, however, are considerably more expensive than Bordeaux mixture when used at the same copper dosage. The object of the present trials was to explore conditions under which they might give effective control of potato blight at reduced copper dosage, and, under these conditions, to compare them with Bordeaux mixture at similarly reduced copper dosage, as well as at other strengths.

A recently discovered sulphur fungicide, Dithane 14, containing disodium ethylenebisdithiocarbamate (Dimond *et al.* 1943), had been found to give satisfactory control of potato blight in Florida (Ruehle, 1944) and in the Lower Rio Grande Valley (Godfrey, 1944) when applied at weekly intervals with a small addition of zinc sulphate and lime to introduce a light flocculum into the spray solution. It was thought that if the necessary number of applications could be reduced by thorough spraying at a few well-chosen dates, this new material might be of some economic interest for potato spraying in England, near industrial establishments, where the action of sulphur gases in the atmosphere on copper deposits may produce phytocidal effects on potato foliage. These effects have not been observed in any of our trials in Devon or Cornwall, but Dithane 14 was included in the 1945 trials in the expectation that at Beaford its fungicidal action against *Phytophthora infestans* would be effectively tested by a severe blight epidemic beginning in July, while at Galmpton a later attack would more closely resemble those most frequently occurring in the northern and eastern counties.

Materials

The complete list of spray treatments included in the trials, with designatory abbreviations and equivalent copper dosages, was as follows:

		% Cu
B	1 % Bordeaux mixture (4.5.40) freshly prepared from granulated copper sulphate and hydrated lime	0·25
½B	½ % Bordeaux mixture (2.2½.40) freshly prepared from granulated copper sulphate and hydrated lime	0·125
¼B	¼ % Bordeaux mixture (1.1¼.40) freshly prepared from granulated copper sulphate and hydrated lime	0·063
¼I	Ialine Colloidal Copper Compound, a colloidal suspension of basic copper carbonate in paste form, containing 12½ % Cu by wt., and used at 5 lb./100 gal.	0·063
¼Bsl	Bouisol, a colloidal suspension of copper oxychloride in liquid form, containing 15 % Cu by wt., and used at 4·17 lb./100 gal.	0·063
¼P	Perenox, a dispersible preparation of yellow cuprous oxide, in powder form, containing 50 % Cu by wt., and used at 1·25 lb./100 gal.	0·063
¼YC	Yellow Cuprocide, a dispersible preparation of yellow cuprous oxide in powder form containing 83 % Cu by wt., used at 0·75 lb./100 gal.	0·063
¼F	Fungex, a cuprammonium compound in liquid form containing 8·75 % Cu w/v used at 5·7 pt./100 gal.	0·063
Di	Dithane 14, a solution containing 3 lb. of disodium ethylenebisdithiocarbamate per U.S. gal. used at 4 pt./100 gal., together with a recommended addition of 1 lb. zinc sulphate and ½ lb. hydrated lime per 100 gal. to introduce a light flocculum into the spray fluid and to improve adhesion	—
Zn	1 lb. zinc sulphate and ½ lb. hydrated lime per 100 gal. (to test for possible fungicidal effect of the addition to Dithane 14)	—
LS	Lime sulphur sp.gr. 1·3, used at 1 in 50	—

In the tables and figure that follow, a + sign after the treatment designation denotes that the material was used with the addition of a calcium caseinate and rosin soap type of spreader and sticker (Ialine Spreader) at 2 lb./100 gal.,

Methods

Difficulties in the assessment of blight damage on the foliage, due to concurrent virus necrosis, were avoided by carrying out the trials at demonstration centres where it was known that good seed had been planted. The nullification of the trials by early onset of potash-deficiency leaf-scorch was guarded against by placing them at centres where extra potash had been applied for demonstration purposes. Suitably even stands of plants for detailed work were secured by making preliminary preparations for trials at a number of centres, but leaving the final choice until the condition of the haulm could be judged.

Two centres were finally chosen, the first at Galmpton near Brixham, where the Majestic plants were very uniform, but small owing to curtailment of nitrogen in the dressing, so that the haulms would retain their individuality to the end of their growing period and not become a tangled mass of prostrate stems on which estimations would be difficult. The second centre was at Beaford, near Torrington, where blight is usually very severe, and where an acre of exceptionally even potatoes (Majestic) was available, with haulm of average size. The seed was not 'A' Certified, as at Galmpton, but good once-grown. Only about 2% of the plants showed symptoms of virus disease (all leaf-roll), and these were uniformly distributed.

The trial plots were randomized in four blocks at each centre. Each plot was subdivided into two for the trial of each copper material with and without added adhesive on adjacent strips. Each individual subplot at Galmpton was 33 ft. long by two drills 25 in. wide, and at Beaford 50 ft. long by two drills 28 in. wide. All the spray fluids were applied by hand, using a knapsack sprayer, and a generous but uniform amount of spray fluid was used to ensure thorough and careful cover of all the foliage. At Galmpton ¾ gal. was used for each subplot on each spraying occasion, and at Beaford 1 gal.

The sprayings were timed, so far as weather conditions would permit, with the object of using the spray fluids of low-copper dosage to the best possible advantage in the light of previous experience. The first sprayings were given as late as it was safe to give them to ensure that the protective deposits were not attenuated by rain before they could serve any useful purpose, and the number of applications was reduced to a minimum, to avoid masking the superiority of the better treatments.

To secure uniform opportunities for infection of the sprayed potatoes from the unsprayed controls all the sprayed plots at each centre were interlaced with unsprayed, in such a way that every sprayed plot was adjacent on one side to an equal area of unsprayed.

The rainfall in each field over the period of trial was obtained by rain gauges placed among the potatoes and read on each date of inspection for disease measurement. Indications of the distribution of rain between inspections were obtained from the daily rainfall totals at the nearest meteorological stations.

The mean expanse of foliage in sq.ft. per plant was estimated on the successive dates of spraying at each centre, by first obtaining the mean weight of the leaf lamina per unit area, and then stripping and weighing the whole of the laminae from representative samples of plants.

The progress of tuber development from the beginning of each trial to harvest was approximately estimated by lifting and weighing the tubers from 10-plant samples in the Bordeaux-sprayed plots on successive dates.

The progress of blight on the sprayed and unsprayed haulms was measured by estimates of percentage decay for all plots on each date of inspection. The methods used for these measurements and for the determination of the final yields were as previously described (Large, 1945). The mean interval between the half-decay dates for sprayed and unsprayed foliage was again taken as the measure of direct foliage protection resulting from treatment.

Estimates of spray retention at both centres were made by methods previously described (Large *et al.* 1946), and the analysis of the many leaf samples, more than 200, were again made by one of us, W.J.B., in the Dartington Hall Laboratory, using an improved type of Spekker absorptiometer for the reading of dithiocarbamate-copper colour densities.

Course of blight attack and protection by spraying

The assessments of blight on the foliage, together with half-decay dates and yields for both trials, are assembled in Table 1, and the corresponding foliage-decay curves, with daily rainfall totals, and the estimated course of tuber development (in plots sprayed with 1% Bordeaux mixture) are shown in Fig. 1.

(*a*) *Trial at Galmpton.* The characteristics of the blight attack are clearly shown by the curve for the unsprayed haulm in Fig. 1(*a*). Blight began in the third week of July, but was kept in check by dry weather until mid-August, when, with rain every day, its progress was rapid, the unsprayed haulm being half dead by 24 August.

The first spraying was given on 10 July, a fine day without rain. The plants were then in flower, but small, of mean height, only 13½ in., and mean foliage area 3·75 sq.ft. The second spraying was given on 31 July, when the plants were 18 in. high, and the mean expanse of foliage per plant was about 5·8 sq.ft. There was no further increase in the expanse of foliage after the date of the second spraying, and the whole expanse was efficiently covered by the second thorough spraying under good conditions. A third spraying was contemplated for 14 August, but on that date the haulms were already just beginning to yellow, and it was evident that the clearly visible deposits on the Bordeaux-sprayed plots would be adequate to protect the haulms until the end of their useful growth.

The two sprayings with lime sulphur and Dithane 14 were clearly insufficient to protect the haulm, giving mean prolongations of growth beyond the unsprayed of only 2 and 4 days respectively, against 20 days with 1% Bordeaux. The ¼ and ½% Bordeaux mixtures did not give such good protection as the standard 1% mixture, but the difference in prolongation of growth was only from 2 to 5 days, at the tail end of the growing period. The proprietary mixtures gave protection of the same order as Bordeaux mixture used at correspondingly low-copper dosage, with the exception of the cuprous oxide sprays ¼P and ¼YC+, which were less effective. The addition of the adhesive made no appreciable difference to the performance of the ¼, ½ or 1% Bordeaux mixtures, but with ¼I and ¼Bsl there was a marked advantage, as both equalled ½B.

The standard error of the mean yield estimates in this trial was fairly low, owing to the very even stand of the plants and uniformity of the soil. The small differences in yield, however, resulting from the few days of difference in prolongation of growth between the different copper treatments, were, as would be expected, less than the significant difference between the yield means. The yields with two applications of lime sulphur or Dithane 14 were not significantly different from those of the unsprayed, while the yields with two applications of all the quarter- and half-strength copper fungicides, with or without added adhesive, were not significantly different from the yield with two applications of 1% Bordeaux mixture, which gave all the protection required.

(*b*) *Trial at Beaford.* The foliage-decay curves, Fig. 1(*b*), show that the conditions of trial at Beaford were entirely different from those at Galmpton. At Beaford blight began on the unsprayed plots in the first week of July; it made little headway until 14 July, but then, with heavy rain on the 15th and during the following fortnight, progress was so fast that the unsprayed haulm was half dead by 27 July, nearly a month earlier than at Galmpton.

At Beaford a few spots of blight were seen in the field on the date of the first spraying, 3 July, and the

TABLE 1. *Results: foliage protection and yield*

Treatment and dates of spraying	Mean percentage of foliage area destroyed or covered by blight lesions							Mean date of half-decay of foliage	Mean prolongation of growth (days)	Mean yield tons/acre
10 and 31. vii	23. vii	31. vii	14. viii	21. viii	6. ix	14. ix	27. ix			
				(*a*) Majestic, 'A' Certificate, Galmpton, 1945						
B+	—	—	—	0·1	6	70	97	13. ix	20	10·4
B	—	—	—	0·1	8	71	99	13. ix	20	10·0
½B+	—	—	—	0·1	6	82	100	11. ix	18	10·3
¼Bsl+	—	—	—	0·3	5	83	100	11. ix	18	9·7
½B	—	—	—	0·1	10	88	100	10. ix	17	9·5
¼I+	—	—	—	0·2	10	89	100	10. ix	17	9·5
¼I	—	—	—	0·2	19	94	100	9. ix	16	8·9
¼Bsl	—	—	—	0·3	9	97	100	9. ix	16	9·7
¼B+	—	—	—	0·3	27	95	100	8. ix	15	9·5
¼B	—	—	—	0·3	31	96	100	8. ix	15	9·3
¼YC+	—	—	—	0·5	60	99	100	5. ix	12	9·1
¼P	—	—	—	0·8	62	99	100	5. ix	12	9·3
Di	—	1	2	5	99	100	100	28. viii	4	8·4
Zn	0·01	2·5	5	22	100	100	100	24. viii	0	7·7
LS	—	1	2	15	100	100	100	26. viii	2	8·3
Unsprayed	0·01	2·5	5	22	100	100	100	24. viii	0	7·7
								Significant difference ($P=0·05$)		1·1
				(*b*) Majestic, good once-grown, Beaford, 1945						
3, 16 and 30. vii	3. vii	16. vii	24. vii	30. vii	13. viii	27. viii	10. ix			
B+	0·01	0·1	0·1	0·1	40	70	98	18. viii	22	—
B	0·01	0·1	0·1	0·1	40	75	99	17. viii	21	14·6
¼I+	0·01	0·1	0·1	0·1	50	85	99	14. viii	18	12·7
½B+	0·01	0·1	0·1	1·0	50	97	99	13. viii	17	11·7
½B	0·01	0·1	0·1	1·0	50	97	99	13. viii	17	11·5
¼B+	0·01	0·1	0·3	0·3	50	95	100	13. viii	17	11·1
¼I	0·01	0·1	0·3	0·5	60	97	100	11. viii	15	12·7
¼B	0·01	0·1	0·3	0·3	60	97	100	11. viii	15	11·0
¼F	0·01	0·1	0·3	1·0	60	97	100	11. viii	15	11·7
¼Bsl	0·01	0·1	1·0	1·0	66	99	100	10. viii	14	12·7
¼P	0·01	0·1	1·0	5·0	70	99	100	9. viii	13	12·6
Di	0·01	0·1	0·3	20	66	98	100	8. viii	12	11·3
LS	0·01	0·2	1·0	33	90	100	100	3. viii	7	11·7
Zn	0·01	0·5	40	66	97	100	100	27. vii	0	—
3 and 30. vii										
B+	0·01	0·1	0·1	5	66	90	100	10. viii	14	—
B	0·01	0·1	0·1	5	75	90	100	8. viii	12	12·0
16 and 30. vii										
¼YC+	0·01	0·5	10	33	80	97	100	4. viii	8	—
¼Bsl+	0·01	0·5	10	33	80	97	100	4. viii	8	—
¼F+	0·01	0·5	10	33	80	97	100	4. viii	8	—
3. vii only										
B and B+	0·01	0·1	0·1	5	80	97	100	7. viii	11	—
¼I and ¼I+	0·01	0·1	0·1	33	90	100	100	3. viii	7	—
Di	0·01	0·1	1·0	50	97	100	100	1. viii	5	—
Unsprayed	0·01	0·5	40	66	97	100	100	27. vii	0	10·5
								Significant difference ($P=0·05$)		2·0

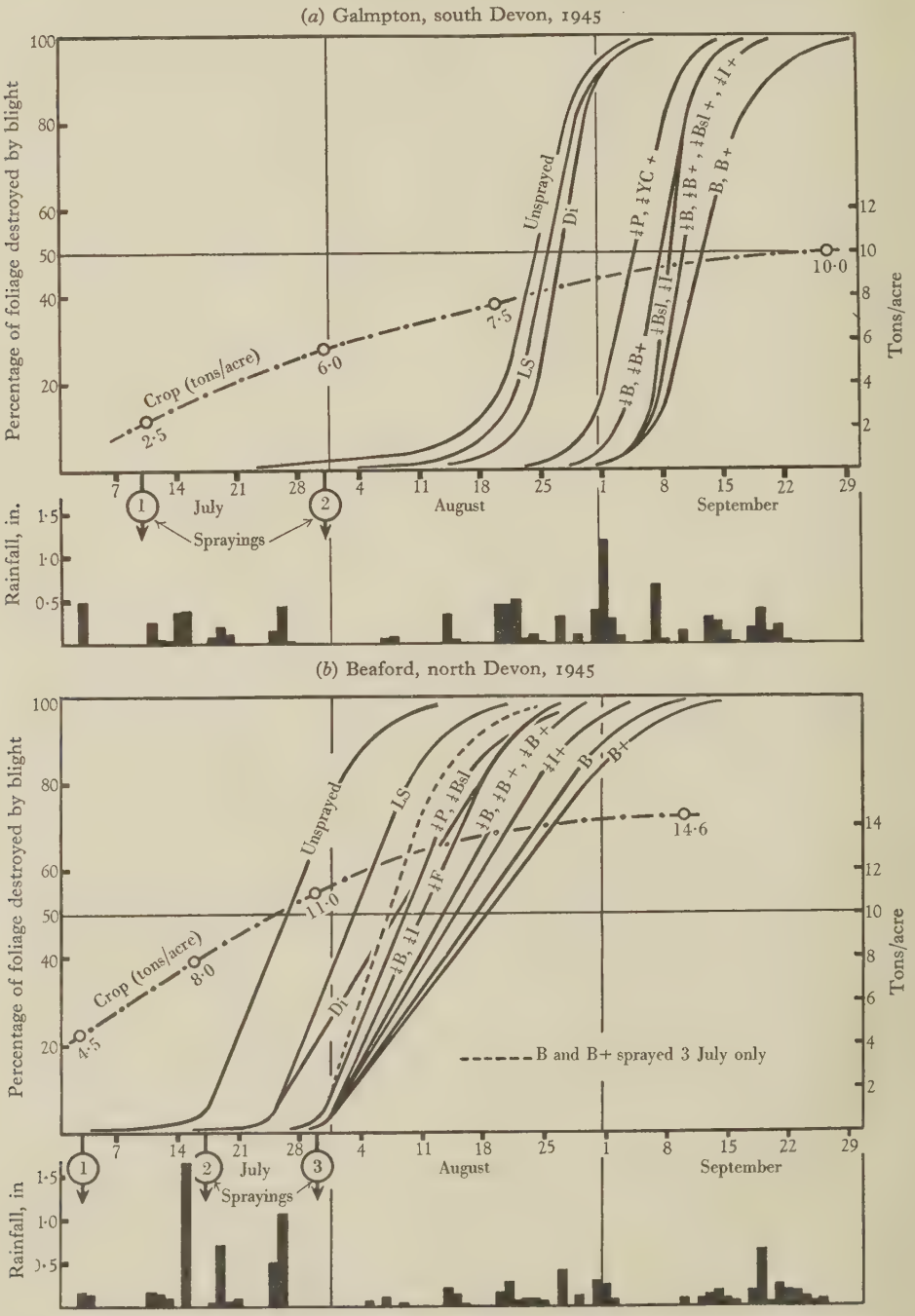

Fig. 1. Progress of foliage decay due to potato blight, in relation to rainfall, dates of spraying, spray treatments, and course of tuber development (in plots sprayed with 1% Bordeaux mixture) at two trial centres in Devon, 1945. Variety: Majestic. For key to treatments, see p. 407.

spraying was done in and between heavy showers. As all the treatments suffered from partial washing off before the deposits were dry, sampling for spray retention had to be deferred until the period between the second and third sprayings, and the first application of three of the treatments, ¼YC+, ¼Bsl+ and ¼F+, had to be abandoned altogether. All the treatments which *were* applied, however, including lime sulphur and Dithane 14, were successful in keeping down the blight infestation to 0·2% or less until 16 July, so that the second spraying, given under good conditions on that date, could be effective. The plants were then 20 in. high, very even, with mean expanse of foliage 7·5 sq.ft. per plant. There was no increase in the expanse of foliage thereafter. A third spraying, again under good conditions, was given on 30 July.

It will be seen from the curves that the third spraying was too late for the two sulphur treatments and also a few days too late for some of the copper treatments, the rapid epidemic increase of blight, represented by the sharp turning up of the characteristic curves, having already begun. Differences in the effects of the several copper treatments in this trial are represented by a conspicuous fanning out of the respective decay curves after the end of July, and by differences in slope persisting over the whole of the month of August.

Once again 1% Bordeaux mixture gave the greatest protection of the foliage, with a prolongation of growth, for three applications, of 21 days beyond the unsprayed, and in this trial the addition of adhesive to standard Bordeaux had a slight positive effect.

For the sake of further comparisons some additional plots were sprayed once only, on 3 July, and others twice only, on 3 and 30 July. The foliage-decay line for plots sprayed once only with 1% Bordeaux mixture is shown dotted in Fig. 1(*b*). The mean prolongation of growth with this single spraying was 11 days.

Reading the results of the whole trial, as regards foliage protection, from the curves, it may be said that three applications of lime sulphur were less effective than a single application of 1% Bordeaux—and that given in the rain. Three applications of Dithane 14 gave rather better protection than this single application of 1% Bordeaux, and about the same protection as three applications of ¼B, ¼F, ¼P, ¼I and ¼Bsl without added adhesive, all of which gave from 13 to 15 days prolongation of growth. The addition of adhesive to ¼I and ¼B in this trial gave distinct improvement, and brought the performance of these two treatments up to equality with ½% Bordeaux mixture, giving about 17 days mean prolongation of growth.

The standard error of the mean yield estimates at Beaford was much higher than at Glampton, owing chiefly to unsuspected variations in soil depth, occurring not only between blocks, but also from plot to plot within the blocks. Thus the yields give hardly any reflexion at all of the real differences in foliage protection achieved by the different treatments. The plots sprayed three times with each of the copper sprays at ¼ or ½ copper dosage, or twice with Bordeaux mixture at full copper dosage, all gave a yield of between 11 and 12½ tons/acre, as compared with about 10½ tons/acre for the unsprayed. The yield with three applications of 1% Bordeaux mixture was significantly higher than that with most of the other treatments.

Spray retention

Spray retention trials for all the copper treatments were made over two test periods, viz. between the first and second sprayings at Galmpton (21 days with 1·44 in. of rain) and between the second and third sprayings at Beaford (14 days with 2·6 in. rain). The leaf samples for initial retention were taken after spraying as soon as the deposits had dried, and there were no losses due to rain between spraying and sampling. Four replicate leaf samples, each of fifty 17·5 mm. diam. disks for each treatment on each sampling occasion, were ashed and analysed separately in the Galmpton trial, while at Beaford there were three such replications. The standard errors of the mean figures for total copper in leaves, and also of the percentage retentions, were obtained by the analysis of variance, and the results at both centres are assembled in Table 2.

The conditions of these two spray retention trials were not very severe. In normal practice with two routine sprayings in Devon and Cornwall it frequently happens that there are intervals of a month between sprayings, with up to 5 in. of rain. But the conditions of these trials could be considered as appropriate and fair for copper fungicides at low-copper dosage which should be applied at more frequent intervals.

Under the moderate conditions of test there was no significant difference in percentage retention between 1, ½ and ¼% Bordeaux mixtures. All showed about 45% retention with or without added adhesive, and the absolute amounts of the copper deposits on the leaves, both at the beginning and at the end of the test periods, were roughly proportional to the copper dosage applied.

None of the proprietary copper fungicides, with or without added adhesive, showed any greater tenacity than Bordeaux mixture at the same low-copper dosage (0·063% Cu), and, indeed, on average between the two trials their percentage retention was about half of that of the Bordeaux mixtures.

At Galmpton, with only 1·44 in. of rain over the test period of 3 weeks, the addition of the caseinate-rosin soap adhesive slightly improved the tenacity of

the spray deposits. But at Beaford, with more rain (2·6 in. in a fortnight), and the initial deposits consisting in part of the more resistant residues from the first spraying, there was no improvement. The slightly superior foliage protection noted at Beaford, where added adhesive was used, may have been due to the lighter rainfall after the third spraying.

that of ¼% Bordeaux mixture. But in agricultural practice, where reliance must be placed on routine sprayings, and the times of application cannot be so nicely chosen, it would seem preferable to select a copper fungicide having the greatest possible resistance to washing off by rain, and to use it at 0·25% copper dosage to secure a margin of safety.

TABLE 2. *Results: spray retention*

	Galmpton			Beaford		
	Mean total Cu in leaves (0·01 mg. Cu/120 sq.cm.) Rain 1·44 in.			Mean total Cu in leaves (0·01 mg. Cu/120 sq.cm.) Rain 2·6 in.		
Treatment	10. vii	31. vii	Retention %	16. vii	30. vii	Retention %
S.E.	+9	+3	+4·6	+5	+5	+5·7
B+	236	105	45	204	137	67
B	228	104	46	174	84	48
½B+	87	41	47	121	56	46
½B	115	49	43	79	38	48
¼B+	48	18	38	70	34	49
¼B	51	21	41	64	33	51
¼I+	81	26	32	70	25	36
¼I	68	13	19	58	25	43
¼Bsl+	89	20	23	66	17	26
¼Bsl	63	6	10	65	21	32
¼YC+	52	10	19	50	14	28
¼P	35	3	9	18	6	33
¼F+	—	—	—	37	10	27
¼F	—	—	—	48	16	33
Significant difference ($P=0·05$)	26	10	13	14	14	16

CONCLUSIONS

In both trials all the copper fungicides used at low-copper dosage gave less efficient protection of the foliage than 1% Bordeaux mixture (5.5.40), and all showed inferior resistance to washing off by rain, both when used alone and with a caseinate-rosin soap type of spreader and sticker.

None of the copper fungicides used at 0·063% copper dosage gave any significantly better protection than ½% Bordeaux mixture (1.1½.40), but it was found that with thorough spraying, and well-timed applications, ¼% Bordeaux mixture itself gave almost sufficient protection for the production of a full crop.

It would appear that in horticultural and gardening practice, where the spraying of a small area can be done thoroughly and at well-chosen times, satisfactory control of potato blight can be achieved by quite a small number of applications of almost any copper fungicide, and with copper dosage as low as

Dithane 14 was more effective against potato blight at 1 in 200 than lime sulphur at 1 in 50, and this was not due to the small addition of zinc sulphate and lime, which when used alone had no effect. But with three applications only, the Dithane 14 treatment gave protection inferior to that obtained with ¼% Bordeaux mixture, and it would appear that with this material, under English conditions as in America, more numerous applications, at weekly intervals, are necessary.

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Further experiments on the control of white rot (*Sclerotium cepivorum* Berk.) in onions, shallots and leeks

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(With 3 Text-figures)

Trials in 1945 confirmed earlier work on the efficacy of mercurous chloride (calomel) for the control of *Sclerotium cepivorum* in spring-sown onions. The application of 1 lb. 4% calomel dust to 50 yd. of seed drill at sowing time gave sufficiently good control of the disease in salad onions. Broadcast application of 1 oz. 4% calomel dust per sq.yd. before sowing was less effective but promising. Post-emergence application of the dust was useless. In autumn sowings, seed-drill treatment at 1 lb./50 yd. controlled the disease in the following spring, but caused severe retardation and loss of crop.

Onion seedlings raised in heat and dipped in an aqueous paste of calomel dust before transplanting showed a high degree of resistance to a severe attack. Similar treatment of shallot bulbs was also effective. In both cases, the treatment substantially increased the yield and somewhat reduced storage losses.

Leek seedlings transplanted into dibber holes did not develop the disease.

INTRODUCTION

This is a continuation of the work reported by Booer (1945) in which it was shown that mercurous chloride applied to the seed drill as 4% calomel dust at sowing time gave a substantial control of white rot in outdoor sowings of spring onions. It was thought advisable to repeat this work and to extend it to onions raised from transplanted seedlings, shallots and leeks. All these crops are popular with smaller growers who, having less ground available for crop rotation, suffer more from the effects of white rot than the large market-gardener or farmer.

The general arrangement of the 1945 trials is shown in Fig. 1. The ground to the right of the line *AB* was that used in the 1943 and 1944 trials. The ground to the left of the line *AB* had not previously carried onions for at least five years. Autumn sowings were in east-west rows, all others being north-south. The whole plot was hand-dug during the winter, and in the early spring 1 oz. of National Growmore Fertilizer 7-7-7 and 1 oz. of sulphate of ammonia per sq. yd. was broadcast and hoed in.

The mycelial and sclerotial stages of *Sclerotium cepivorum* were observed on both spring- and autumn-sown onions on 24 May, which is an early date for the disease in this country.

ONIONS

(*a*) *Autumn sowings*

Five rows each 28 yd. long, 15 in. apart, were sown with onion seed, var. White Lisbon, on 29 August 1944. The centre row was untreated, and the remaining four rows were treated with 4% calomel dust at the rate of 1 lb. of dust per 50 yd. of drill. The drill was opened, the dust applied as evenly as possible, the seed sown and the drill was closed.

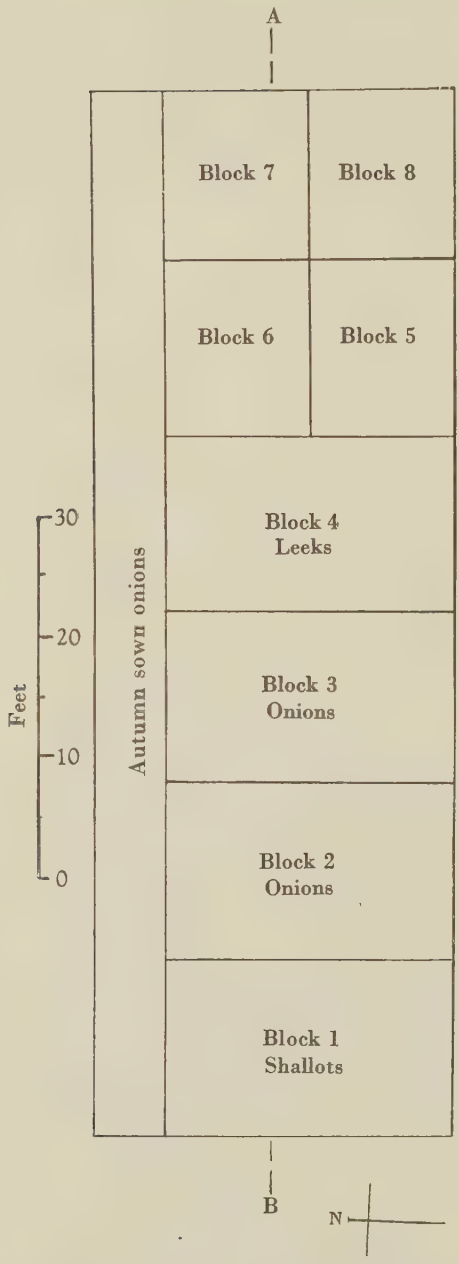


Fig. 1. General arrangement of plots.

Considerable retardation of the plants was observed in the treated rows, and the entire plot appeared to suffer badly from winter frosts, giving an uneven distribution of plants. As the retardation persisted well into the spring, periodical counts were made and the mean weights of the plants determined. The results given in Table 1 show the mean number of plants per foot and the mean weight per plant determined at various dates by pulling strips 3 ft. wide across the plot at random.

TABLE 1. *Autumn sowings—mean populations per foot and weights per plant*

	Untreated		Treated	
	Plants/ft.	Mean wt./ plant/g.	Plants/ft.	Mean wt./ plant/g.
4 May	7·7	5·3	10·7	3·9
11 May	12·3	8·9	10·3	7·3
18 May	9·3	16·1	9·7	12·3
25 May	18·7	13·8	9·3	17·5
1 June	8·3	20·6	10·0	20·1
8 June	9·3	36·3	7·3	31·2
15 June	6·3	39·4	7·3	30·7
Means	10·3	—	9·2	—

This trial was discontinued after 15 June, partly because the plants exceeded the size for saleable salad onions, and also on account of a severe attack of *Heterosporium allii* var. *cepivorum*. Visual examination of the plants remaining showed about 5% white rot in the untreated rows and none in the treated rows.

The lack of uniformity in this trial is shown in the plant population figures in Table 1, and the effect of losses by disease is completely masked. The figures for the plant weights, however, show that the treatment causes serious retardation and consequent loss of crop.

The conclusion reached from this experiment is that even with an unusually early attack, the treatment of autumn sowings is not justified, in spite of the fact that the disease is effectively controlled. The loss of crop would only be justified by special considerations, such as the desirability of completely controlling the disease in a small allotment or self-contained market garden.

(*b*) *Spring sowings*

Trials were made to check the results obtained in 1944. Blocks 5, 6, 7 and 8, as shown in Fig. 1, were sown with seed var. Valencia on 28 March, the rate of sowing being 4 g./24 ft. row, equivalent to 16 lb./acre at 12 in. row spacing.

In blocks 5 and 7 the following treatments were applied to alternate rows immediately before sowing:

A. Control.

C. 1 lb. 4% calomel dust per 50 yd. drill.

D. 1 lb. 4% calomel dust per 25 yd. drill.

The dosage of 1 lb. 4% calomel dust per 100 yd. drill, constituting treatment B in the 1944 trial was omitted as obviously inadequate.

There were five randomized replicated rows 12 ft. long for each treatment, and the observations which were made on various dates consisted of counts of the entire rows. The results in Table 2 give the mean populations in plants per foot.

TABLE 2. *Spring sowings—seed drill treatment—mean populations per foot*

	Block 5 Treatments			Block 7 Treatments		
	A	C	D	A	C	D
24 May	15·2	17·7	17·5	17·9	19·4	19·4
12 June	10·6	17·5	19·2	15·8	20·1	20·5
25 June	6·9	18·3	17·9	13·5	19·7	20·5

The results show that although the infection was heavier in block 5 than in block 7 treatment C was quite effective in both cases, and treatment D gave results which were not significantly better, thus confirming the 1944 conclusion that a dosage of 1 lb. of 4% calomel dust per 50 row yd. is adequate for spring sowings of salad onions. The plants having reached the maximum permissible size, the experiment was discontinued. No insect pest or disease other than white rot was observed.

Blocks 6 and 8 were used to study alternative methods of application, block 6 being devoted to broadcast application and block 8 to post-emergence application of the dust.

Block 6 was divided into four plots 6 ft. square, two of which were not treated and two were treated immediately before sowing, with 1 oz. 4% calomel dust per sq.yd. lightly raked in. This rate of application is equal to 1 lb. of dust per 50 yd. of drill at 12 in. row spacing. The plant populations given in Table 3 are the means of ten replicates expressed in plants per foot.

TABLE 3. *Spring sowings—broadcast treatment—populations per foot*

	Treated	Untreated
24 May	18·6	18·8
12 June	18·3	16·4
25 June	17·4	13·3

If the results in Table 3 are compared with those for the adjacent block 7 in Table 2, it is seen that the losses in the untreated rows were similar. Although the broadcast treatment gave a measure of control of the disease it was not so effective as the localized seed-drill treatment. The broadcast method, however, deserves further investigation with heavier doses, since it has obvious advantages for the larger grower.

In block 8 no treatment was applied until the presence of the disease was confirmed on 24 May. On 25 May alternate rows were treated at the rate of 1 lb. of 4% calomel dust per 50 yd. of row, the dust being applied to the soil as close as possible to the line of the plants. The results in Table 4 are the means of six replicated rows each 12 ft. long and are expressed as plants per foot.

TABLE 4. *Spring sowings—post-emergence treatment—populations per foot*

	Treated	Untreated
13 June	9·3	6·1
25 June	6·7	3·7

As the rate of sowing was the same as in blocks 5, 6, 7 and 8, it is evident that in block 8 the disease was very severe. Post-emergence treatment in these circumstances is quite useless.

Through the courtesy of a number of amateurs, trials were made in various parts of the country on the seed-drill treatment. Although no figures were forthcoming, the general conclusion was that the treatment gave good results, notably at Boston and Bourne (Lincs), Newark (Notts), Wrexham (Denbigh), Swindon (Wilts), Yeovil (Som.), Newtownards (Co. Down) and Churton (Cheshire).

Two exceptional results are worth recording. In a trial at Croydon (Surrey) no white rot was observed, but there was a severe attack of onion fly (*Hylemyia antiqua*) which reduced the population from 11·4 to 3·1 plants per ft. between 22 April and 1 July. In the rows which had been treated in the drill with 1 lb. of 4% calomel dust per 50 yd. no control of onion fly was observed. A trial at Northampton was reported as a complete failure. An examination of the defective plants showed that no white rot was present, but there was a heavy infestation of eelworm (*Anguillulina dipsaci*). Treatment of the seed drill with 4% calomel dust had given no control of this pest.

(*c*) *Transplanted seedlings*

Block 3 was used for a trial with healthy seedlings raised under glass. The variety was Ailsa Craig; the seed was sown on 29 January and the seedlings pricked off into standard 3 in. trays, sixty plants per tray, on 21 February. On 2 May the seedlings were carefully pulled from the trays and dipped, approximately to the base of the free portion of the leaves, in a paste prepared from 480 g. of 4% calomel dust and 210 ml. of water. The seedlings varied in size between 5 and 9 mm. diameter at the bulb and took up a mean of 5·11 g. paste per plant. The dipped seedlings were immediately planted at 6 in. intervals in rows 14½ in. apart. At this spacing the treatment was equivalent to the application of 18½ lb. mercury per acre. There were forty-nine plants per row, with

five rows each treated and control rows alternately. In the control rows the seedlings were similarly treated with a paste made from the filler used in the 4% calomel dust.

No effect of the disease was visible until mid-July when the first symptom in infected plants was yellowing of the bottom leaves. This effect was quickly followed by death of the plant. The distribution of dead and obviously diseased plants, determined by visual inspection on 17 September is shown in Fig. 2.

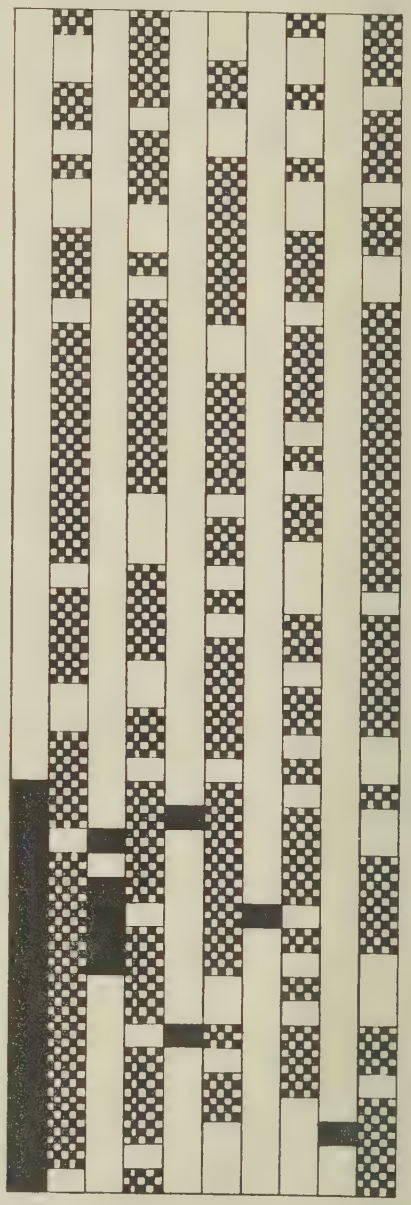
The bulbs were lifted on 19 September, and the results obtained are shown in Table 5.

TABLE 5. *Details of crop from transplanted seedlings*

	Treated	Control
Bulbs per 100 seedlings planted:		
Sound	88·1	25·7
Diseased	4·1	18·4
Dead	7·8	55·9
Yields per 100 seedlings planted (lb.):		
Sound	46·5	11·2
Diseased	1·9	6·1
Weights per bulb (g.):		
Sound	240	197
Diseased	218	150

The results show that the treatment was highly effective in controlling a severe attack of white rot and the benefit of the treatment is reflected in the crop. The equivalent yields of sound onions per acre are, 14·9 tons from treated rows, and 3·6 tons from control rows. A contribution towards the additional yield arises from the greater weight per bulb in the treated rows. Although the difference fails to reach statistical significance ($n=8$, $t=2{\cdot}21$) it should be noted that a similar result was obtained with shallots, as reported later in this paper.

Before dealing with the effects of storage, mention should be made of an unsuccessful experiment in block 2. This was designed as a trial on transplanted autumn sowings and was intended to compare such transplants with the glasshouse seedlings used in block 3, with respect to both the incidence of white rot and the effect of the calomel paste treatment. The variety used in block 2 was Bedfordshire Champion, the seed being sown outdoors on 6 September. The plants were removed from the seed bed on 2 May, treated with 4% calomel dust paste as already described, and planted at 6 in. intervals in rows 14½ in. apart. There were forty-nine plants per row with five rows each treated and control rows alternately. The mean consumption of paste was 4·19 g. per plant. Probably owing to the late transplanting date, all the seedlings failed to make appreciable growth. The trial was ignored and the ground became covered with weeds. Autumn rains and mild weather caused the surviving onions to outgrow the weeds and a count on 24 September



Diseased plant in untreated row

Diseased plant in treated row

Fig. 2. Distribution of diseased onions.

gave mean results of 62·5 plants in treated rows and 49·5 plants in control rows per 100 seedlings planted.

The sound onions from block 3 were put into store, the bulbs from each row being put into a separate 'Dutch tray' and the whole stored in a cool, well-ventilated room free from frost. Periodical examinations were made and the results given in Table 6 require the following explanations. The figures for sound, useless and sprouted bulbs and for the yield are the means calculated on the basis of 100 seedlings originally planted. Useless bulbs included those that were obviously decayed, and those that were too shrivelled to be saleable. Sprouted bulbs were those which, after removing the sprout, could be sold, and the net weights of such bulbs are included in the yield figures on the assumption that these onions would be sold immediately after the examination. The percentages of useless and sprouted bulbs respectively are calculated on the corresponding numbers of bulbs originally put into store.

An examination of the useless bulbs collected during November showed that the chief cause was premature and multiple sprouting, the mobilization of food reserves causing a general collapse of the bulb. Some neck rot was present although not the chief cause of loss, and occasional instances of rot caused by *Penicillium* sp. were observed. In subsequent examinations most of the losses were caused by premature sprouting.

As the variety Ailsa Craig is not usually regarded as a long keeper the experiment was discontinued on 1 February. The results in Table 6 show that the calomel paste treatment had a beneficial effect on storage losses, and attention is again directed to the consistent advantage in the mean weight per bulb in favour of bulbs from treated rows.

Table 6. *Effect of storage losses on onions var. Ailsa Craig*

(Treated rows in ordinary type—control rows in italics)

	Examination dates				
Per 100 seedlings planted:	27 Sept.	30 Oct.	30 Nov.	1 Jan.	1 Feb.
Sound bulbs	88·1	84·5	71·5	66·2	63·2
	25·7	*24·9*	*18·0*	*15·9*	*14·3*
Useless bulbs	—	3·6	6·9	4·5	1·6
	—	*0·8*	*3·7*	*1·2*	*1·2*
Sprouted bulbs	—	—	5·3	0·8	1·2
	—	—	*3·3*	*0·8*	*0·4*
Yield (lb.)	46·5	42·9	38·2	35·5	34·2
	11·2	*10·3*	*8·9*	*8·3*	*7·7*
% useless bulbs (totals)	—	4·2	12·0	17·0	18·8
	—	*4·8*	*20·4*	*22·2*	*26·8*
% sprouted bulbs (totals)	—	—	6·0	6·9	8·3
	—	—	*14·3*	*15·9*	*17·5*
Equivalent yield, tons/acre	14·9	13·8	12·3	11·3	11·0
	3·6	*3·3*	*2·9*	*2·7*	*2·4*
Wt. of sound bulbs/g.	240	231	227	224	220
	197	*187*	*203*	*203*	*200*

Leeks

Seedlings of leek var. Musselburgh, raised in sterilized soil until about 10 in. high, were transferred to block 4 on 27 July and put into dibber holes about 8 in. deep. In accordance with popular practice the holes were not filled in, and in this instance no water was applied. An excellent crop was obtained and no white rot developed in spite of the fact that the ground was heavily infected. It is of interest to record that although white rot is not uncommon on leeks, a disease-free crop was raised by this method on this occasion.

Shallots

In block 1 shallots, variety English Red, were planted on 1 March. Rows 15 in. apart were opened with a triangular hoe, and the bulbs placed 12 in. apart. There were twenty-five bulbs per row, six rows being planted with treated bulbs, and the alternate five rows with control bulbs. The treatment consisted of dipping the lower half of the bulb in the 4 % calomel dust paste already described. The bulbs took up a mean weight of 4·5 g. of paste per bulb, and the treatment was equivalent to the application of 8 lb. of mercury per acre. The control bulbs were similarly dipped in a paste prepared from the filler only.

Until mid-June the disease produced no visible effect, but thereafter serious losses occurred in the control rows, the first symptoms being similar to those observed in transplanted onions, namely, yellowing of the lowest leaves followed by death of the affected bulbs, on a number of which the presence of *Sclerotium cepivorum* was confirmed. On 13 July the clusters were lifted to sever the roots and to hasten ripening. An examination was made on 2 August, the distribution of diseased clusters being shown in Fig. 3, and analysis of the results given in Tables 7 and 8.

TABLE 7. *Shallots produced per* 100 *bulbs planted*

	Treated	Control
Sound bulbs	763	424
Soft bulbs (not apparently diseased)	36	105
Infected bulbs	23	119
Dead bulbs	2	46
Total bulbs	824	694

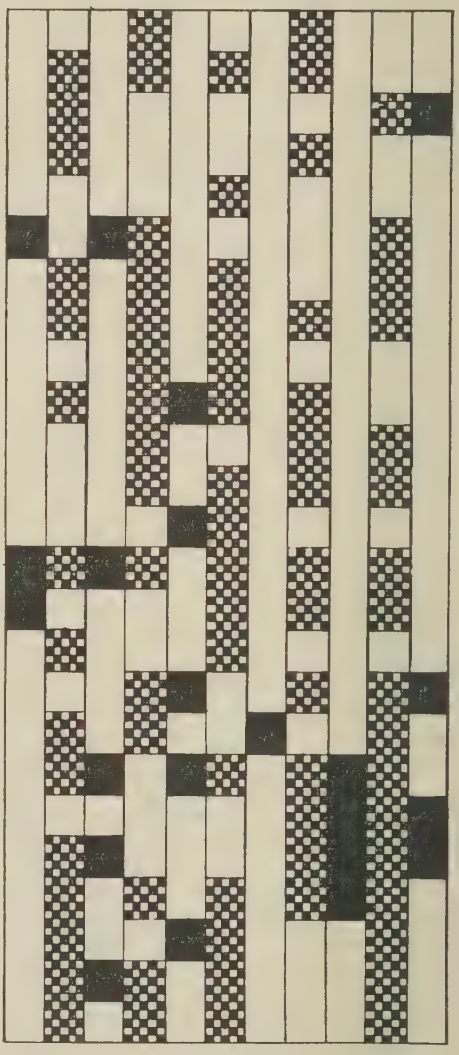
TABLE 8. *Analysis of disease in shallots*

	Treated %	Control %
Disease-free clusters	85·4	38·4
Clusters with 1 diseased bulb	9·3	19·2
Clusters with 2 diseased bulbs	4·0	11·2
Clusters with 3 or more diseased bulbs	1·3	31·2

In Table 7 all differences between the results from treated and control rows are significant at $P=0{\cdot}01$ or less, and attention is particularly directed to the increase in total bulbs resulting from the treatment. As no clusters were lost, it is clear that the treatment resulted in increased multiplication of the original bulb. The extent to which this may be due to disease control is discussed later. Also since no clusters were lost, the percentage figures given in Table 8 are strictly comparable, showing that the treatment has been highly effective.

The sound and soft bulbs were stored in the same manner and in the same store as that used and already described for onions. The results of periodical examinations are given in Table 9 in which the same method of expressing the results is used as in Table 6, except that as the sprouted shallots were also useless, their weights were not included in the yield figures. The results in column *X* show the net figures after removing 25 bulbs per replicate for seed, following the examination on 1 February. This is not only normal practice, but is justified by the fact that twenty-five bulbs per replicate were brought into the experiment at the beginning. Their removal thus gives the figures for the crop available at the period when it is normally required.

An examination of the useless bulbs collected during November showed that although losses from neck rot and *Penicillium* sp. were somewhat higher than in onions, the primary cause again was premature sprouting. The results in Table 9 also show



Diseased plant in untreated row

Diseased plant in treated row

Fig. 3. Distribution of diseased shallots.

that the calomel treatment had a slightly beneficial effect on storage losses, and it will again be noted that the bulbs from treated rows were consistently heavier than those from untreated rows.

TABLE 9. *Effect of storage losses on shallots*

(Treated rows in ordinary type—control rows in italics)

	Examination dates						
	19 Sept.	1 Oct.	1 Nov.	1 Dec.	1 Jan.	1 Feb.	*X*
Per 100 bulbs planted:							
Sound bulbs	799	728	657	605	583	558	458
	529	*447*	*397*	*370*	*356*	*332*	*232*
Useless bulbs	—	71	71	47	21	21	—
	—	*82*	*50*	*18*	*13*	*18*	—
Sprouted bulbs	—	—	—	3	2	4	—
	—	—	—	*8*	*2*	*6*	—
Yield (lb.)	50·8	45·9	39·2	35·6	33·4	31·1	28·0
	30·0	*25·6*	*22·1*	*19·9*	*18·7*	*17·4*	*14·2*
% useless bulbs (totals)	—	9·0	18·0	23·7	26·3	28·9	—
	—	*15·0*	*25·0*	*28·3*	*30·6*	*34·2*	—
% sprouted bulbs (totals)	—	—	—	0·4	0·6	1·1	—
	—	—	—	*1·5*	*1·9*	*3·0*	—
Equivalent yield tons/acre	8·0	7·1	6·1	5·5	5·1	4·8	4·4
	4·6	*4·0*	*3·4*	*3·1*	*2·9*	*2·7*	*2·2*
Wt. of sound bulbs/g.	29·0	28·5	27·5	26·7	26·0	25·3	32·4
	25·6	*26·2*	*25·2*	*24·5*	*23·9*	*23·7*	*27·7*

GENERAL DISCUSSION

It should be noted that in the 1945 trials, as in those of the two previous years, there is no instance of the disease being completely controlled. The application of calomel to the soil results in alleviation rather than cure. Garrett (1944), describing the parasitic activity of root disease fungi, underlines 'the essential fact that infection is a "struggle" between the offensive forces of the fungus and the defensive forces of the root'. My results support this view, and point to the conclusion that the action of calomel, which decomposes into mercury in the soil, as shown by Booer (1944), is to exert an influence on the struggle between the pathogen and the host, in some manner favourable to the latter.

The results provide quantitative information on the extent to which the crop benefits from an alleviation of the damage due to white rot. In the case of shallots (Table 7) the multiplication of the original bulb is increased, the proportion of soft bulbs reduced, and the mean weight per bulb is increased. Onions show an increased bulb weight, and in both cases the improved condition results in reduced storage losses. Conversely, the conclusion indicated is that even in mild infections, resulting in comparatively few losses by death, the unseen crop lost in the struggle with *Sclerotium cepivorum* might be quite serious.

Information on the spread of the disease is derived from the fact that the northern half of the plot had carried no species of *Allium* for many years. The distance travelled in one season was about five yards, but the fact that the disease appeared simultaneously in autumn- and spring-sown onions suggests that the mechanism was the distribution of resting sclerotia by cultivation.

The trials that have been made provide the small grower with means to combat white rot. The problem of the larger grower still has to be solved, but the preliminary results of the broadcast application of calomel provide the basis for further trials.

The author is indebted to Mr W. C. Moore, M.A., Ministry of Agriculture Plant Pathology Laboratory, Harpenden, for his diagnosis of the causes of storage losses. The help of Mr F. M. Wyatt and Miss B. Skinner, B.Sc., in recording the field trials is also gratefully acknowledged.

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Distribution of violet root rot (*Helicobasidium purpureum* Pat.) of sugar beet and preliminary experiments on factors affecting the disease

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(With Plate 12)

Examination of sugar beet grown in eastern England over the period 1936–43 has shown that up to 5 % of factory tarehouse samples contain roots infected with violet root rot. The results of the survey suggest that there are considerable areas where light attacks of the disease occur, as well as fields in which severe attacks develop when susceptible crops are grown. The disease occurred on roots grown in many types of soil but most frequently on those from light alkaline soils. It was found to reduce the sugar content and increase the dirt tare of sugar beet.

The causal fungus, *Helicobasidium purpureum*, has a wide host range and observations suggest that it persists in the field on weeds, as severe attacks on cultivated crops often occur in weed-infested fields. It also survives as sclerotia, which develop freely on the roots of susceptible crops. While the undisturbed soil conditions of leys and uncultivated land favour increase in infectivity, good cultural practices, trap cropping and application of nitrogenous fertilizers act as a check. These control measures will reduce infectivity even when applied to soil in which consecutive, susceptible crops are grown. Wet soil conditions did not favour the development of infection, and the frequent occurrence of the disease in wet places may be due to such soil conditions favouring survival of sclerotia.

Experiments with compost made from factory waste showed that although the fungus can survive the process, its virulence was so reduced that there is no likelihood that use of such compost would lead to severe violet root rot infection.

Introduction

Helicobasidium purpureum causes a root rot of numerous herbaceous plants, shrubs and young trees. Among farm crops its chief hosts are carrots, sugar beet, potatoes, swedes, clover and lucerne, and individual cases of severe attacks on these crops have been recorded in England and Wales (Moore, 1943). It has generally been considered that the disease is confined to isolated patches of ground and is not, therefore, of much practical consequence (Petherbridge & Stirrup, 1935), but there is some evidence that it is becoming more serious, not only in sugar beet, but also in other crops (Moore, 1943, p. 16). The extent of the disease fluctuates with the season. In 1938, for instance, it was more widespread than in most years, both in England and in Europe (Decoux *et al.* 1939).

The appearance of the disease and the relationship between host and parasite have been described in detail by several authors (Prillieux, 1897; Faris, 1921). In the early stages, mycelial strands extend along the exterior of the root; the tissues are then penetrated by hyphae from small sclerotium-like infection cushions known as 'corps miliaires', and the infected root is later enveloped in a dense felt of mycelium. The host tissues are killed where they are penetrated by the fungus and, although the rot may be comparatively shallow at first, the extent is often considerably increased on account of secondary invasion by other organisms. *H. purpureum* is not found in active growth in the soil apart from its host, and may therefore be classified as a soil invader (Garrett, 1938).

In common with other diseases of this type, the incidence of violet root rot depends upon factors affecting the rate of mycelium development and upon the amount of infective material in the soil (Garrett, 1944). The disease is initiated in a crop by infective material surviving in the soil, the amount of which is determined by the extent of infection in previous crops and by soil and cultural factors affecting sclerotium production and survival. The persistence of the fungus on the roots of perennial weed hosts is also of importance.

The behaviour of the organism in pure culture has been investigated by Buddin & Wakefield (1924), who associated the perfect stage (*H. purpureum*) with the mycelial stage, known as *Rhizoctonia crocorum*, but little work has been recorded on the incidence and control in the field of the root diseases it causes. Various authors, in describing outbreaks of violet root rot, have placed emphasis on different soil, cultural and climatic factors affecting the disease. Gleisberg (1932) states that rhubarb receiving a well-balanced fertilizer escaped attack. Van der Lek (1917) found indications that manures rich in nitrogen favoured the disease. In the opinion of Peyronel (1939) the development of the fungus is favoured by high water content of the soil, richness in organic

material and acidity. On the other hand, the fungus was very prevalent on highly calcareous clay-loam soils in Texas in 1929 (Dana & Wolff, 1938), where it was also favoured by ample moisture. Similarly, dewberries (*Rubus flagellaris*) in low-lying damp situations in California were killed or stunted (Poole, 1931) by the fungus.

Duggar (1915), in his summary of early literature, points out that while several authors have emphasized the occurrence of the disease in wet situations, others have reported it in the driest situations permitting crop growth. Buddin & Wakefield (1924) acknowledged that moisture may play an important part in affecting the activities of the parasite, but considered that it is the high optimum temperature for growth of the fungus (25·5° C.) which explained the geographical distribution of severe attacks in the field. This is in accordance with the recorded distribution of the disease in England, where it occurs mainly in the east, south and south-west (Moore, 1943), and its absence from Scotland (Dennis & Foister, 1942).

From these reports it is apparent that under varying conditions of soil, cropping and climate, different factors predominate in determining the degree of severity of the disease.

Occurrence in the field

Survey. Satisfactory field surveys of this disease during the growing season are difficult to make, because there are, usually, no obvious symptoms on the aerial parts of infected plants. The disease is apparent on the lifted crops, but small lesions are easily overlooked owing to adhering soil, and only occasionally is it possible to examine the lifted crop over a whole field at once, as the roots are usually carted away when they are ready.

A convenient method of determining the incidence of the disease is to examine the washed samples of beet which are taken from each load on arrival at the beet-sugar factories for determination of tare and sugar content. The data given in this section have been so obtained. Arrangements were made for the tarehouse workers to pick out and mark all diseased beets from the samples so that they could be traced to the farm where they were grown. The roots were later examined by the authors and the numbers affected with various diseases recorded.

A washed sample was only about $\frac{1}{4}$% of a load, and thus many slight outbreaks of the disease were not detected. This is clear when it is realized that one diseased root in a sample represents about 4% infection in the load.

The proportion of samples containing roots infected with violet root rot at the Brigg, Bardney and Kelham sugar-beet factories over a number of years and the number of growers to whom they were traced are shown in Table 1. The figures were obtained by examining samples from all the loads entering each factory during the season, the number of which varied from 20,000 to 40,000.

TABLE 1. *Incidence of violet root rot in tarehouse samples of sugar beet*

	1936	1937	1938	1939	1940	1941	1942	1943
Brigg factory								
Percentage of samples containing violet root rot	0·9	1·5	2·8	2·4	4·7	5·0	2·5	4·0
No. of growers	—	165	305	301	405	445	—	—
Bardney factory								
Percentage of samples containing violet root rot	—	1·5	3·4	2·1	4·8	—	—	—
No. of growers	—	187	282	226	460	—	—	—
Kelham factory								
Percentage of samples containing violet root rot	—	0·9	0·9	0·3	0·6	—	—	—
No. of growers	—	41	73	35	42	—	—	—

At Bardney and Brigg factories, which draw their beet mainly from Lincolnshire, the proportion of samples containing violet root rot varied annually from 0·9 to 5·0%; at Kelham infection varied from 0·3 to 0·9%. The major part of this infection was traced to the western part of Lincolnshire, and comparatively little to those parts of Nottinghamshire and Derbyshire from which the factory draws most of its beet. Table 2 gives the proportion of infected samples taken from beet which was diverted to the Lincolnshire factories in 1940 from four other factory areas: Bury St Edmunds, Ely, Cantley and King's Lynn, which draw their beet from Norfolk, west Suffolk and Cambridgeshire. These figures are based on the examination of comparatively few samples, but they suggest that there was a similar degree of infection to that found in Lincolnshire.

The proportion of samples containing different numbers of roots infected with violet root rot at Brigg factory in 1940 is given in Table 3. Similar figures for the same factory over a period of 5 years are summarized in Table 4, where the average

number of infected roots in the samples containing violet root rot are shown. The majority of samples contain only one diseased root, and only a small proportion of samples contain three or more diseased roots. This suggests that there are large areas having light infection with occasional fields more severely affected. The average number of infected roots per sample varies each year in a similar manner to the proportion of infected samples (Table 1).

TABLE 2. *Incidence of violet root rot in tarehouse samples of sugar beet in* 1940

Factory area	No. of samples examined	Percentage of samples containing infected roots
Bury St Edmunds	715	1·8
Cantley	2614	5·5
Ely	1628	4·6
King's Lynn	74	5·3

The average number of roots traced annually, over the five years 1937–41, to the various parishes of Lincolnshire has been divided by the average acreage of beet grown in the parishes, and the resulting 'infection ratios' tabulated according to the predominant soil type on which beet is grown in the parish. This is not a strictly accurate procedure because of the variety of soils encountered in individual parishes, but it does serve to illustrate the general picture obtained when the infections are plotted on the map. The results are shown in Table 5. In general, crops on the light soils showed the heaviest infection and those on the heavy soils the least. The heaviest infection occurred in crops grown on the limited area of warp‡ in north Lincolnshire. Crops grown on the soils overlying the Oolitic limestone were heavily infected, particularly where the soil was light. Many of the worst infections in crops on sands and loams occurred where the soil

TABLE 3. *Intensity of infection found in tarehouse samples of sugar beet—Brigg factory,* 1940

No. of infected roots per sample ...	1	2	3	4	5	6	7	8 and over (highest = 20)
Percentage of infected samples	80·8	10·7	4·1	2·0	0·9	0·5	0·4	0·6

TABLE 4. *Extent of infection found in tarehouse samples of sugar beet*

	Av. no. of infected roots per diseased sample				
	1937	1938	1939	1940	1941
Bardney	1·15	1·33	1·12	1·41	1·70*
Brigg	—	1·22	1·18	1·43	1·36

* Only the worst infected roots and samples were recorded in this case which gives a high value for this figure.

TABLE 5. *Violet root rot infection on various soil types in Lincolnshire based on infection traced to parishes in* 1937–40 *inclusive*

Predominant beet acreage on	Average infection rating†
Warp	0·101
Limestone	0·088
Sand	0·056
Loam	0·049
Black fen	0·039
Wold	0·031
Strong fen	0·020
Heavy loam	0·019

† Average infection rating $= \frac{\text{number of roots traced per year}}{\text{average beet acreage}}$.

Distribution on soil types

Sugar beet is grown on most of the soils occurring in Lincolnshire, though predominantly on the medium and lighter soils. Violet root rot has been traced to fields in most districts where beet is grown and is distributed on all soil types.

was overlying chalk, or in valleys where chalky debris was incorporated in the soil. On the other hand, beet in the high fields on the top of the chalk hills, referred to as Wold in Table 5, was not so heavily infected. Crops on the lighter black fen soils were more severely infected than those on the stronger fen soils, but in general they were not so severely infected on the fen and alluvial lands of south Lincolnshire as on the higher lands. The lowest infection rating occurred on clay-loam and fen soils. Occasionally, however, individual cases occurred of heavy infection on clay soils. This was in contrast with the incidence of the disease on the lighter soils, where there were well-defined districts in which severe infections occurred persistently every year.

In 1939, all the growers whose beet had shown heavy infection in the tarehouse samples were interviewed, and when the infection could be traced to a single field, soil samples were collected and *p*H

‡ Warp is the term used for land which has been subjected to deliberate flooding by rivers with the object of obtaining a deposit of silt.

determinations made on them. The values obtained, classified according to soil types, are shown in Table 6. Twenty-eight out of thirty fields examined had a *p*H between 7·1 and 8·5, and they were all light or medium soils. Two fields of sandy soil were acid. This evidence, that severe infections occur mainly on alkaline soils, has been confirmed by field observations over several years.

Host range

The host range of the fungus is very wide. Duggar (1915) gives a list of hosts and Mr W. C. Moore of the Ministry of Agriculture's Plant Pathology Laboratory has kindly given access to a collection of references to the incidence of the fungus on 169 species distributed in forty-three families. Recent work has shown that the fungus does not exhibit narrow host specialization. Ogilvie *et al.* (1935) found by experiment that *Helicobasidium purpureum* attacking asparagus can also attack carrot, sugar beet, mangold, parsnip and potato. Buddin & Wakefield (1927) made cross-infections of the fungus from sugar beet on to carrots and clovers, and from potato and mangold on to various clovers. During the present investigation, cross-infections with carrot and beet isolates have frequently been made, and infections on weeds have been found in tubs of soil which have been inoculated with isolates from carrot and sugar beet. Field observations have shown that where one crop is attacked in one year, different crops are attacked in the following years. Weed hosts growing amongst diseased cultivated host plants are also attacked. Amongst those on which it has been found in sugar-beet fields are: *Lychnis alba* Mill., *Sonchus oleraceous* L., *Achillea Millefolium* L., *Geranium molle* L., *Euphorbia Helioscopia* L., *Mentha arvensis* L., *Matricaria Chamomilla* L., *Capsella bursa-pastoris* Medik. and *Senecio vulgaris* L.

Sclerotia are produced on the roots when the disease is in an advanced stage (Pl. 12, fig. 2*a*, *b*). The fungus can be found also in the mycelial stage during the winter months on the stolons and persistent roots of perennial plants such as *Mentha arvensis* and *Achillea Millefolium*. Severe attacks in cultivated crops are often associated with weed infestation, and the persistence of the fungus on weed hosts is considered to be a major factor in initiating such outbreaks.

The presence of infection cushions of *Helicobasidium purpureum* and dead host tissue on these plants leaves no doubt that the fungus has penetrated and there is a true parasitic relationship, but other cases are found where the subaerial mycelial felt, which frequently extends in the autumn along the surface of the ground from infected tap roots, has merely enveloped and not attacked nearby weeds. It is possible that this epiphytic relationship has sometimes been mistaken for parasitism. For instance, although the fungus has frequently been found enveloping *Agropyron repens*, no pentration has been observed, whereas Baudys (1930) has reported this plant to be a host. *Stellaria media*, reported as a host by Eriksson (1913), has been seen growing in profusion amongst infected crops but never itself attacked.

Effect on the crop

Plants affected with violet root rot are sometimes recognized during the growing season by wilting of their foliage or, if the disease is severe, there may be patches where the plants appear shorter than neighbouring healthy plants. Normally, however, the disease is not noticed until the roots are lifted. Violet root rot does not appear seriously to affect the yield of the crop unless infection is heavy. Diseased roots have an abnormal amount of adhering soil, which is very difficult to knock off, with the result that the dirt tare is increased. This is an important addition to costs where the crop has to be transported long distances, and the individual grower runs the risk of an exceptionally heavy dirt tare deduction from his load if the sample contains more than a representative number of infected roots. The extent to which diseased roots are rotted when freshly lifted varies considerably; they may be largely decomposed, or the rot may be only about 1 cm. deep, even though the exterior of the root is completely enveloped in mycelium. Determinations of dirt tare and sugar content on two freshly dug samples (28 lb.) are shown in Table 7. The dirt tare is almost doubled and the sugar content is decreased by 1–2 %, but there is still enough sugar in diseased roots to justify sending them to the factory to be processed.

Diseased roots decompose quickly under favourable conditions. Bacterial and fungal decomposition starts in the tissues penetrated by *Helicobasidium purpureum* and quickly spreads to the other parts of the root. If a crop containing more than about 10 % diseased roots is clamped, it begins to heat within a fortnight in mild weather, leading to severe losses. Small numbers of affected roots in clamps are harmless as, although they normally decompose to a greater or lesser extent, the rot does not spread to the other roots during the time of clamping necessitated by conditions in this country (up to 2½ months), nor is there danger of overheating. Occasionally clamps are found where the mycelium of *H. purpureum* has spread to parts of the root which were not affected while the plant was growing, but such growth is too slow to be of practical importance.

Sclerotia

The sclerotia of *H. purpureum* were described by Duggar (1915) as rounded or flattened, deep violet-brown bodies, thickly clothed with a persistent velvety felt and varying in diameter from a few

millimetres to several centimetres. He found them in contact with diseased saffron bulbs, also in the angles of the larger branches of alfalfa roots and in the soil round them, but not frequently in connexion with diseased carrots, beet or potatoes. Faris (1921) described them as reddish black bodies, longer than they are wide and varying from pin-head size to 5 mm. in length. They were produced within the superficial mantle of mycelium on the surface of potato tubers and stems.

During the present investigation sclerotia have been found in profusion on all severely attacked roots of both cultivated crops and weeds. They appear to be essentially a development from a well-nourished mycelium in an advanced stage of attack on plants, and are not found at all in connexion with light or early infections.

Frequently in the centre of the sclerotium there is a cavity in which the remains of the vascular elements of the root can be found. The epidermal tissues and the original 'corps miliaires' may be found on the surface of some sclerotia. Sclerotia are sometimes formed in a similar way on the surface of the swollen tap root, verrucose, sclerotial masses, normally elongated and flattened but otherwise of indefinite shape, being embedded in the mycelial strands.

Some preliminary observations were made on the incidence of sclerotia by washing soil samples. The soil was sieved to remove gravel and stones and shaken with water for 5–10 min. It was then sieved through wire gauze (28 meshes to the inch) and the silt washed away under the tap. The residue was placed in a large dish into which a gentle stream of water was run to wash away the light organic matter,

TABLE 6. *Violet root rot infection in relation to pH of soil*, 1939

	*p*H range				
	5·3–5·9	7·1–7·5	7·6–8·0	8·1–8·5	Total
Soil type	No. of fields				
Limestone and chalky loam	—	4	4	2	10
Sand	2	1	1	3	7
Warp and alluvial	—	—	—	6	6
Loam	—	—	—	4	4
Carr and fen	—	3	—	—	3
Total	2	8	5	15	30

TABLE 7. *Effect of violet root rot on the dirt tare and sugar content of sugar beet* 1937

Sample	Dirt tare (lb./cwt.)	Sugar content (%)
Healthy	8	16·6
Diseased	13	15·5
Healthy	12	16·7
Diseased	22	14·7

On carrots the sclerotia are not usually directly attached to the tap root but are found on the finer lateral roots. They are of fairly regular shape, cylindrical with rounded or tapered ends, up to 1 cm. in length and 3–4 mm. diameter. On sugar beet, although sclerotia are found all over the surface of the tap root, they tend to be concentrated in the grooves where the laterals are situated. They are very variable both in size and shape; when formed on finer lateral roots they may be similar to those produced on carrots, or the whole root may be converted into a long curled sclerotium, which easily breaks up. On larger lateral roots irregularly shaped sclerotoid masses are formed. Sections of these show that the sclerotial tissue is produced from the mycelium which has grown in the cortex of the root and the remains of the cortex cell walls may be seen.

and was then examined under a lens. The sclerotia were easily distinguished from other materials in the soil by their shape, colour, texture and attached mycelium. Some sclerotia, which appear normal at first, disintegrate easily into brown particles when crushed; these were discarded as decomposing. Live sclerotia have a characteristic hard gelatinous texture. They are differentiated into an outer dark layer and a whitish grey core, and when crushed in water turn it milky.

During the winter, soil samples were collected with a $\frac{1}{2}$ in. auger from various depths in a tub of clay soil in which infection had occurred on carrots for a number of years. In samples of about 5 cu.in. taken at different depths, thirteen sclerotia were detected in that from the top 4 in., seven sclerotia were found between 4 and 8 in. and none between 8 and 12 in. Of three similar sets of samples of about 5 cu.in. taken from infected sugar-beet fields, two yielded no sclerotia, but in the third there were eight sclerotia in the top 6 in. and two between 6 and 12 in.

The vertical distribution of sclerotia in the soil may be compared with that of other organisms. Leach & Davey (1938) report that 80% of the sclerotia of *Sclerotium rolfsii* are in the upper 6 in. and less than 2% below 12 in.; in the case of

Phymatotrichum omnivorum, King & Hope (1932) found sclerotia distributed between 12 and 24 in. These were most numerous between 16 and 20 in. under cotton, between 6 and 30 in. but mostly between 12 and 18 in. under alfalfa and between 6 and 48 in. under a chinese elm.

It was shown in the following experiment that sclerotia occurring in both deep and shallow layers of the soil are effective as inoculum. Surface scrapings from heavily infected sugar beet were buried at various depths in tubs of soil in which sugar beet was subsequently sown. In the tub in which the inoculum was placed at the base, 14–15 in. below the surface, two roots remained healthy and two were severely infected; where the inoculum was placed 7 in. below the surface, all five roots were infected, and where the inoculum was incorporated in the surface 2 in. of soil three roots were infected and one remained healthy. There was similar variation in the extent and distribution of the lesions on the roots with all three treatments.

Experimental methods and materials

Field experiments were arranged on land where there was natural infection. The experimental plant was sugar beet, which was grown in accordance with normal agricultural practice. The amount of infection was determined by counting the roots showing lesions, either by examining the crop in the field when harvested, or after washing the plot produce. In the smaller experiments, such as Exp. 2, each root could be examined carefully in the field, but in the larger experiments, where greater numbers of roots were dealt with (about 20,000 in Exp. 1), only a more cursory examination could be made.

For laboratory and small-scale experiments, the fungus was obtained in pure culture from infected carrot and sugar beet by planting out infection cushions picked from roots after surface sterilization (Buddin & Wakefield, 1924). Cultures were maintained on 3 % malt-extract agar.

Experimental work on the disease has been restricted by failure to develop a technique giving measurable infections quickly enough for use in the laboratory or in small containers in the glasshouse. No infection was obtained on the radicles of beet seedlings grown from seed placed on agar-culture inoculum in sand or soil in tumblers, after 11 days in an incubator at 25° C. or after 4 weeks in the glasshouse. Using the same culture, beet plants with tap roots of about $\frac{1}{4}$ in. diameter were potted up with agar inoculum adjacent to the root. The plants were kept from February to June in a glasshouse, which was heated at first, and small lesions were then observed. Neuwirth (1932) reported similar failure to infect seedlings, but found that 4–5-months-old plants were readily infected.

Several further experiments using agar inoculum placed adjacent to carrot and sugar-beet tap roots, and potato tubers potted in soil in a heated greenhouse in the winter months, proved unsatisfactory and gave little or no infection.

Owing to the unsatisfactory results obtained with agar inoculum, the following trial was made. Surface scrapings from infected sugar beet were mixed with soil and placed in two boxes, 6 in. deep, which were sown with carrots and sugar beet respectively. After 3 months in the glasshouse (April–June) it was observed that infection had occurred, and when the plants were examined 1 month later, five carrots were covered with mycelium, four had lesions on the side, two had tail lesions and one remained healthy. Only one out of twelve sugar beet showed infection. As carrots grow satisfactorily in a confined space and appear to be more susceptible, they are preferable to sugar beet for experiments of this type.

The method adopted for small-scale experiments, therefore, was to grow stump-root carrots in the open from May till the autumn, in pots 8 in. in diameter. The appropriate soil was placed in the pots to a depth of $3\frac{1}{4}$ in., the inoculum placed on top of this and then a further 4 in. of soil added. The inoculum consisted of sieved debris from infected sugar beet which had been collected from infected crops during the previous autumn and stored in an outhouse. Approximately 60 c.c. was added to each pot.

The seedlings were thinned to about twenty per pot and the soil surface treated with 4 % calomel dust to prevent carrot-fly attack.

Where seed was sown on 26 May infection was apparent on the roots by 10 August (Exp. 3) but was not sufficiently advanced to assess by the visual grading method. Infection was adequately advanced for quantitative assessment by the middle of September.

The variation in position and shape of the lesions produced on the roots made the quantitative expression of the extent of the infection difficult. Measurements and weighing were found to be unsatisfactory because the roots which were obviously most severely attacked were often stunted and rotted. A visual method of scoring was adopted. Carrots showing a range of degrees of infection from 0 = no infection to 10 = complete envelopment of the root, were preserved and used in each experiment as standards (Pl. 12, fig. 1).

Experiment 1

This experiment tested the effect on the disease of crop rotation over 8 years. In 1938 a field was found at Scopwick, Lincs, showing relatively even, light infection on 6 acres. This area was divided into three 2-acre strips on which three different crop rotations were arranged, viz.:

	Strip A Continuous susceptible crops	Strip B No susceptible crops for 6 years	Strip C Normal rotation	Plots nos. 30 and 31
1938	Beet	Beet	Beet	Beet
1939	Barley	Barley	Barley	Barley
1940	Seeds	Barley	Barley	Barley
1941	Beet	Turnips	Beet	Beet
1942	Barley	Barley	Barley	Derelict
1943	Seeds	Rye grass	Seeds	Derelict
1944	Beet	Wheat	Wheat	Wheat
1945	Beet	Beet	Beet	Beet

The crops were fertilized and grown according to normal agricultural practice. The 'seeds' consisted of a commercial mixture of red and white clovers, alsike, trefoil and rye grass and was undersown in the barley crops, thus providing the annual sequence of susceptible crops on strip A. The produce was mown for hay in June and grazed with sheep in the autumn. Each year all strips had an indigenous population of susceptible weeds, but these were not excessive and no special precautions were taken to eliminate them. In 1941, sampling plots 30 and 31 on strip C came within the wire of a military defence position. The 1941 crop was harvested, but these two plots then remained derelict until 1944.

On each strip there were twelve evenly distributed $\frac{1}{40}$ acre sampling plots, which could be located each year by sighting from permanent posts around the margins of the field. Each time there was a beet crop on one or more of the strips the roots from the sampling plots were examined in the field. In 1941, 1944 and 1945 a sample of 40–120 roots was taken from the heaps of roots on each plot which had been passed in the field examination as *not infected*, and re-examined after washing. A considerable number of small lesions were found on these roots. Counts on washed samples showed that the field examination accounted for only 57% of the total infection. The percentage infection found after washing was highly dependent on the field count, which suggests that a constant proportion of infected roots was missed in the field. The estimated percentage infection is subject to large variations, as the number of infected roots in washed samples is very small. For these two reasons, and also because no washed samples were examined in 1938, the field counts have been used in the analysis and presentation of the results.

As is usual with variable data of this type, the square roots of the percentage infection have been used in the analysis; by this means the bias of very divergent readings is reduced. The results of the analysis and the field counts are shown in Table 8.

Under all three rotations there was a significant reduction of infection in the 1945 beet crop compared with that of 1938. The least reduction was on the strip which had been continuously under susceptible crops and the greatest on that where there had been no susceptible crops, but the difference in the reduction between these two strips did not reach significance. Judged by the change on strips A and C between 1938 and 1941 and on strip A between 1941 and 1944, the decrease in infectivity of the soil was progressive over the period of the experiment. The increase of infection in the second of two consecutive beet crops on strip A in 1944 and 1945 does not reach significance. In contrast with the decrease on the cultivated strips there was a large and significant increase in infectivity on plots 30 and 31 during the period when they were derelict though, while cultivated between 1938 and 1941, they showed the same tendency towards reduction in infectivity which was characteristic of the rest of the experiment. Between 1941 and 1945 infection on plots 30 and 31 increased from 0·5 to 8·6%, thus reaching a considerably higher value than at the beginning of the experiment.

When the experiment was started in 1938, the field had just changed ownership. This involved a change from shallow horse ploughing and close rotation to deep, power ploughing and more adequate cultivations and fertilizer applications. The results of the experiment suggest that some factors involved in the cultivation of the land reduced soil infectivity, and that this effect outweighed even continuous cropping with susceptible crops.

Experiment 2

At Hackthorn, Lincs, in 1942, natural infection with violet root rot occurred in a fertilizer experiment which was initially arranged to test the effect of beet-yellows virus on yield, by infecting the plants on one-half of split plots. Violet root rot determinations were made on both halves of the split plots, so that any effect of the virus infection on its incidence would be the same for all fertilizer treatments.

The following fertilizer treatments were used:

0·8 cwt./acre nitrogen as sulphate of ammonia (20·6% N).

1·0 cwt./acre P_2O_5 as superphosphates (16·5% P_2O_5).

1·2 cwt./acre K_2O as muriate of potash (60% K_2O).

5 cwt./acre agricultural salt.

20 tons/acre farmyard manure.

The farmyard manure was applied on 10 March and the whole experimental area was then ploughed. The agricultural salt was applied on 19 March and the other fertilizers on 13 April.

The treatments were applied in factorial arrangements to thirty-two plots in four blocks. The farmyard manure was applied to strips of plots so that its main effect was confounded with block differences. The plots each contained about seventy plants, 11 in. apart in five rows, 18 in. apart, with guard plants and pathways all round the plots. The soil was a limestone loam in a moderate state of fertility. Yield responses were obtained in the field only to sulphate of ammonia and dung. Sugar-beet seed of variety Sharpe's Klein 'E' was sown on 15 April.

depressed infection in the first year by 30%, and in the second year by 4%. The effect in the first year reached significance but, as it is based on only duplicate strip treatments, it is not well determined. The increase in infection produced by salt in 1942 was not significant, but salt depressed infection significantly in 1943. In that year it delayed germination considerably, so it is possible that its effect on infection was indirect. There was no significant effect of either superphosphate or muriate of potash in either year.

Experiment 3

This experiment tested the effect of various soil conditions on the development of violet root rot on

TABLE 8. *Effect of crop rotation on the incidence of violet root rot in sugar beet* (*Exp.* 1)

Year	Strip A Continuously under susceptible crops: Mean sq. root of percentage infection		Strip A Continuously under susceptible crops	Strip B No susceptible crops 1939–44 inclusive	Strip C (excluding plots 30 and 31). Normal rotation	Plots 30 and 31, as strip C but derelict 1942–3
			Mean percentage infection			
1938	2·197	±0·133	5·7	6·3	4·5	1·8
1941	1·802		3·9	—	1·0	0·5
1944	1·023		1·2	—	—	—
1945	1·248		1·7	0·6	0·4	8·6
Mean reduction in sq. root of percentage infection between 1938 and 1945			0·949	1·660	1·394	−1·680
Standard error			0·264	0·264	0·289	0·646

TABLE 9. *Effect of fertilizer treatments on violet root rot infection in sugar beet* (*Exp.* 2)

	Effect on percentage violet root rot infection: 1942		1943	
Sulphate of ammonia	−4·12	±1·72	−1·75	±0·99
Superphosphate	+0·38		+0·88	
Muriate of potash	+2·75		−1·38	
Agricultural salt	+1·88		−2·75	
Organic material	−4·75 ± 0·98		−0·25 ± 3·95	
Mean infection (%)	13·62		6·38	

The experiment was repeated in 1943, the same fertilizer treatments again being applied to the same plots. A heavy dressing of beet tops was applied in December to the strips which were treated with dung in 1942, while the other strips were cleared of beet tops before ploughing. Each year, when the plots were harvested, the roots were examined for violet root rot infection; the results are shown in Table 9.

The mean infection dropped from 13·6% in 1942 to 6·4% in 1943. Sulphate of ammonia caused a reduction in infection of 26% in 1942, and 24% in 1943, but this effect just failed to reach significance in the second year. The organic manure dressing

carrots grown in pots, using the method described on p. 425. The treatments were applied in 3 × 2 × 2 factorial arrangement in six blocks of six pots (Yates, 1937). The treatments were:

Clay soil from Saxby, Lincs, *p*H 7·0.
Sandy soil from Saxby, Lincs, *p*H 7·0.
0·5% by weight of hydrated lime added to the soil.
0·25% by weight of sulphuric acid added to the soil.
Soil maintained wet by keeping the pots in saucers of water.
Soil maintained rather dry.

The addition of lime and sulphuric acid was made to the soil about 6 weeks before the experiment was begun. Both original soils had a rather high free calcium carbonate content, with the result that the sulphuric acid had a negligible effect on *p*H; it had, however, a pronounced 'saddening' effect on both soils, the clay with added acid becoming very intractable. The lime addition raised the *p*H of both soils above 8.

The pots were inoculated with the fungus and sown with stump-root carrots on 26 May 1944. Most of the seed in the pots of soil to which lime was added failed to germinate, so the top inch of soil was removed and replaced by the original untreated soil

and fresh seed sown in this on 12 June. Half the roots were harvested in September, leaving the remaining roots evenly distributed in the pot to be harvested in December. The infection on each root was graded by the visual scoring method.

The pots of soil were kept through the winter in an unheated glasshouse. Soil samples, collected from ten places in each pot with a ½ in. auger, were examined for sclerotia. In the spring of 1945 a light dressing of artificial fertilizer was applied to each pot. They were sown with carrots on 29 May. This year free drainage was provided for all pots. The roots were lifted and graded according to the extent of infection on 26 November. The results of the 1944 and 1945 observations are shown in Table 10.

In 1944, the extent of infection of the carrots in clay soil was rather greater than in sandy soil, but the difference did not quite reach significance. The carrots grown in wet soil were significantly less infected than those in the drier soil. This was probably caused by restricted aeration, since the carrots showed definite stunting due to waterlogging. The lime treatment prevented the carrots from germinating at first and severely stunted those which did grow; infection developed on only one of these plants, and therefore pots receiving this treatment have been omitted from the calculations. The sulphuric acid treatment tended to reduce infection at the 13 September sampling, to a less degree at the December sampling, and not at all in 1945.

From the point of view of determining the treatment effects there was no advantage in sampling in December compared with September, although the amount of infection had almost doubled in that time. In 1945 the overall amount of infection was greater, and the same treatment effects were evident but with less marked differences.

The sclerotium population in the wet soil was somewhat lower than in the dry, but the other treatments had little effect. The sclerotia in the limed soil were almost certainly those added as inoculum, but were evidently not viable since they produced no infection in 1945, when the carrots made better growth than in the previous year.

TABLE 10. *Effect of soil conditions on the incidence of violet root rot in carrots (Exp. 2)*

	Mean infection grading (max. = 10)			No. of sclerotia per 2 kg. soil
	13 Sept. 1944	19 Dec. 1944	26 Nov. 1945	Feb. 1945
Clay	2·8	4·9	8·5	36
Sand	1·7 ± 0·40	3·6 ± 0·46	7·0	41
Wet	1·2	2·7	7·2	32
Dry	3·3 ± 0·40	5·8 ± 0·46	8·3	45
Sulphuric acid	1·6	3·9	7·8	34
Untreated	2·9 ± 0·40	4·5 ± 0·48	7·7	36
Lime	Nil	Nil	Nil	7
Mean (excluding lime)	2·2	4·2	7·8	37·3

Experiment 4

A pot experiment was arranged to determine the effect of the removal of a susceptible crop at an early stage on the infection which develops in the following year. Since sclerotia are found only on roots in an advanced stage of the disease, it was thought possible to reduce the sclerotium population of the soil by inducing them to germinate, and then removing the susceptible crop before more had been produced.

The pot technique described on p. 425 was used. Stump-root carrots were sown on 26 May in inoculated soil which consisted of 1 part clay and 1 part sandy loam. The following treatments were applied to sixteen pots in a 4 × 4 Latin square arrangement:

Fallow.

Carrots removed early—23 August 1944.

Carrots removed later—12 February 1945.

Carrots dug in—12 February 1945.

Some of the infected carrots had rotted by 12 February, but as far as possible all diseased material was picked out of the pots concerned.

In 1945 a light dressing of artificial fertilizer was applied to each pot and carrots sown on 29 May. The results obtained when the roots were harvested and the infection graded on 12 October are given in Table 11.

Early lifting depressed infection compared with the fallowed pots in which considerable infectivity persisted. Heavier infection resulted where the first crop was lifted late or dug in than where it was lifted early or where the pots were left fallow, but there was no difference between the infection following late lifting and that following digging in the diseased crop.

Experiment 5

Observations on the infection developing on carrots or sugar beet grown in tubs of different types of soil were made throughout the 5 years 1939–43. The containers were 5 gal. galvanized iron oil drums, with a drainage hole at the base. A single container was used for each soil type. The soils were collected from arable fields in Lincolnshire. In April 1939 sugar beet were sown in the tubs, and at the beginning of May, when the plants were singled, a piece of *Helicobasidium purpureum* culture on agar was placed against the tap root of each plant. In each subsequent year the crop was sown in April or May and harvested in October or November, but no further infective material was added. Either three to five sugar beet or about twenty carrots were grown in each tub. The tubs were kept in the open, and the soil was not disturbed throughout the period except to cultivate the top 6 in. with a trowel at the time of harvesting and sowing. A dressing of compound fertilizers was applied each year. The tubs were watered during the summer when necessary. The presence or absence of infection on individual roots was observed, and the average degree of infection noted as slight, moderate or severe.

In the winter 1943 of the sclerotium content of soil samples from the tubs to a depth of 1 ft. was determined. The observations are summarized in Table 12.

In the first year there was heavy or moderate infection of plants grown in all soils except the clay; on these plants the lesions were small, and there was obviously poor mycelial development. In each of the following years, however, they were most severely infected. It seems probable that the clay soil favoured the survival of sclerotia and that the severe attack was due to the large amount of infective material remaining in the soil. Marked infectivity persisted in the wold soil and in the sand (1), while in the acid, light fen and acid sand (2) it remained moderate.

Infectivity practically disappeared after 2 years in the garden loam and after 1 year in the Digby Fen soil. On both these soils a very light infection occurred subsequently in one year, no doubt due to surviving, slow-germinating sclerotia.

These results may be compared with the behaviour of the pathogen in the field in these soils. The Digby Fen soil was collected from a field with severe natural infection in the low-lying wet places. This natural infection on a field carrying a reasonable rotation suggests that the wet conditions favoured sclerotium survival, whereas when the soil was well drained in the tub, even with continuous cropping with a susceptible crop, infectivity soon died out. The garden soil was taken from the site of an experiment where reduced infection was obtained on susceptible crops in consecutive years following the addition of inoculum to the field plots (Table 13). Although the soil is obviously favourable for mycelial growth and the development of the disease, and is known to support the production of sclerotia when infection is heavy, it does not favour the survival of the pathogen from year to year.

TABLE 11. *Effect of date of lifting a susceptible crop on subsequent soil infectivity* (*Exp.* 4)

		Treatment in 1944				
	Mean	Fallow	Carrots lifted early	Carrots lifted late	Carrots dug in	S.E.
Violet root rot infection: average grading in 1945	5·49	5·46	2·83	7·04	6·65	±0·23

TABLE 12. *Violet root rot infection on crops in various soil types* (*Exp.* 5)

	*p*H	1939 Beet *Inf. grad.	1940 Beet Inf. grad.	1941 Carrots Inf. grad.	1942 Carrots Inf. grad.	1943 Carrots Inf. grad.	1944 Carrots Inf. grad.	Sclerotia in 1943 (winter)
Clay, Billinghay	7·0	8+	10+++	10+++	10+++	8+++	10+++	12
Wold, Cabourne	8·1	10++	10+++	10+++	8++	Not sown	2+	12
Garden, Hackthorn	7·4	10+++	6+	0	0	1+	0	0
Light fen, Digby	6·6	10+++	0	1+	0	0	0	0
Light fen, Eastville	5·9	6++	4+	7++	8++	3+	7++	1
Sand (1), Snitterby	6·6	8++	10+++	10+++	10+++	Not sown	3+	6
Sand (2), Snitterby	6·4	10+++	7++	2+	2+	Not sown	0	0

* All infections adjusted to a basis of ten plants. Actual number of beets were three to five and carrots about twenty.

\+ Light infection. ++ Moderate infection. +++ Severe infection.

Experiments 6A, B *and* C

Organic refuse consisting of lateral roots of sugar beet and weeds is sometimes converted into compost at the factories and then applied to agricultural land. Three experiments were carried out to determine whether there was danger of spreading violet root rot infection by this practice.

The following materials were used:

Compost (A) from an experimental heap of 108 cu.ft. made at Kelham Factory.

Compost (B), made by the Kelham Factory during 1939–40.

Compost (C), made by the Brigg Factory during 1938–9.

TABLE 13. *Violet root rot infection in crops grown in Hackthorn garden loam* (*Exp.* 5)

	1940 Sugar beet	1941 Carrots	1942 Carrots	1943 Sugar beet	1944 Carrots
	Percentage of roots infected				
Duplicate plots	27·4	5·6	5·2	3·3	2·7
	16·1	6·3	1·5	4·3	1·7

The material used for compost A consisted of 30 cwt. 'tailings' mixed with 10 cwt. chopped, infected roots collected from the field. 30 gal. of liquid pond lime, containing 150 lb. $CaCO_3$, was added and the heap turned over several times. The mixture was then heaped and trodden down in a frame of railway sleepers without a roof. Sulphate of ammonia was added to each 1 ft. layer during heaping, the whole clamp receiving a total of 1 cwt. The dimensions of the completed heap were 4 ft. × 4 ft. 6 in. × 6 ft. high. At regular intervals readings were taken of the temperature at the centre of the heap, and at a depth of 6 in. from the centre of one side. The maxima recorded were 47 and 56° C. respectively.

The heap was made on 22 December 1939. On 14 May 1940 a sample of the compost was applied to two small plots at the rate of 15 tons/acre. Chopped, diseased roots were applied at approximately the same rate to two further plots, while two plots were left untreated as controls. The material was dug in and the plots were sown with sugar beet, each plot being left with about 100 plants after singling. When the crop was lifted on 14 October no diseased roots were found on the control plots or those receiving compost, but forty-seven infected plants were found on those to which diseased roots had been added. It was concluded that the ground was free from the fungus at first, and although it favoured the development of the disease if a suitable inoculum was used, no infection of the beet resulted from the addition of compost. In the following year carrots also remained free from infection on the compost and control plots.

Tests using the same compost were also carried out in 6 in. pots. Six control pots were filled with soil only, and twelve filled with soil with which a heavy dressing of the compost had been incorporated. Half of the pots of each treatment were sown with carrots and half with sugar beet on 16 May, and all were kept in the open during the period of the experiment. The soil was free from the fungus as no disease developed in the control pots. Five of thirty-six carrots grown in the soil plus compost were diseased, but infectivity was not sufficiently virulent to affect the sugar beet.

Compost B was made on a large scale with factory waste using burnt lime but no sulphate of ammonia. In 1940 the compost was applied at the rate of 15 tons/acre to three strips of land, each approximately $\frac{1}{3}$ acre. The land was sown with sugar beet and all the roots examined in October, but no violet root rot infection was found.

Compost C was also made on a large scale with factory waste, one lot with the addition of pond lime only and the other with pond lime and sulphate of ammonia. The compost was applied to $\frac{1}{30}$ acre plots at the rate of 20 tons/acre. When the sugar beet grown on the plots was examined on lifting, only one infected root was found in the whole experimental area, and that was on a plot which had received compost made with pond lime only.

These results indicate that there is no likelihood of causing severe violet root rot infection by applying compost made from factory waste, but there is a possibility that the fungus may survive the composting process and so initiate foci of infection.

DISCUSSION

There are two aspects of the control of violet root rot on farm crops. First, there are the measures to be taken in fields where the disease is severe in order to reduce infectivity to a level at which a susceptible crop may be grown without the risk of heavy losses. Secondly, in view of the widespread distribution of the disease in arable districts, it is necessary to know what practices will keep it in check, and conversely those which will lead to its increase.

There is no doubt that in favourable circumstances close rotation of susceptible crops can lead to heavy infestation of the soil. Some of the worst infections observed in sugar-beet crops have occurred where there was a carrot-sugar beet rotation. On the other hand, there is little evidence that cropping is a major factor in the incidence of the disease. In all the severe cases detected in the tarehouse examination in 1937, the succession of crops over the previous 5 or 6 years was traced where the individual field of origin could be ascertained. Out of sixteen fields in

all, one had carried three susceptible root or clover crops, seven fields two and six fields one. One field had been continuously under cereals for 4 years and one was ploughed-up grassland. One of the fields was reputed to have had a four-course rotation involving barley, turnips and 'seeds' since the land was enclosed. Thus infection cannot be attributed solely to growing susceptible crops frequently in the rotation. Furthermore, in the experiments described in this paper there are three instances in which the amount of infection has fallen in consecutive years on land continuously under susceptible crops. It is apparent therefore that there are factors involved in the cultivation of the fields which reduce the infection by an amount which will outweigh the increase incurred by growing susceptible crops.

As *H. purpureum* has a wide host range it is not always possible to judge the presence or absence of host plants in a field by the recorded cropping, since the weed population may supply as great an abundance of susceptible plants as a cultivated crop. The frequency with which severe outbreaks of the disease are associated with weedy fields suggests that indigenous plants are an important factor in building up a high level of infectivity. A primary necessity in control is to keep the field free from susceptible weeds, particularly those perennials with persistent roots and stolons, which will inevitably perpetuate and increase the infective material in the soil.

It is a general observation that severe infection is not found in crops grown in fields which have been well cultivated and are in a high state of fertility. The way in which this may arise is illustrated by the results of Exp. 1. The field used for this experiment changed ownership in 1937. It had previously been kept in a very moderate state of fertility and never ploughed more than 7 in. deep, but since 1937 it has been ploughed deeper and adequately fertilized. Infection in the beet crop dropped significantly between 1938 and 1944 or 1945 even on those plots cropped continuously meanwhile with susceptible crops. When the rapid increase in infectivity which took place on the uncultivated plots is compared with the general decline in infectivity on the cultivated plots, it is apparent that the cultural practices involved in growing crops on arable land have a profound effect on the disease. This may be due partly to the control of weed hosts and partly to a reduction in the time of survival of sclerotia. The conditions favouring the survival of sclerotia of similar root-disease fungi are those of undisturbed, moist soil of low aeration, whilst disturbance by cultivation, high microbiological activity and periodical drying have the opposite effect (Garrett, 1944). There is much indirect evidence that this is so with the sclerotia of *H. purpureum*. They are known to be produced to a depth of 12 in., and consequently the normal horse ploughing of 5–6 in. depth may leave considerable numbers undisturbed. The undisturbed conditions of grassland also evidently favour the accumulation of considerable amounts of infective material.

Sclerotia are removed from the soil either by becoming non-viable and decomposing or by germinating and being dissipated as mycelium. This process may be accelerated by growing a trap-crop of a susceptible plant and then removing it while the fungus is still in the mycelial stage and before it has produced fresh sclerotia. The success obtained in this way in Exp. 4 justifies the trial of this measure in the field. Success will depend on the thoroughness with which the trap crop is lifted, for, if considerable pieces of root or infected plants are left in the soil, it is probable that growth of the fungus will continue on them. Thorough cultivation of the soil after the crop has been removed should assist in reducing this possibility. The control of weeds will tend to reduce sclerotium population in a similar way to trap cropping, if susceptible weeds are removed in an early stage of development.

The normal sanitary practice of removing diseased roots when a crop is lifted is an obvious precaution to avoid returning infective material to the soil, but it is doubtful whether special efforts in this direction are justified, for, when the disease has reached an advanced stage, it is inevitable that a great part of the sclerotium-bearing tissue is left in the soil. This is illustrated by the results obtained in Exp. 4, where it is apparent that the amount of infective material present in the soil is no longer a limiting factor, because there is no difference in the extent of infection occurring in pots from which the crop had been removed when the disease was in an advanced stage and that in the pots where all the infective material had been dug in. If diseased roots are discarded in the field, however, there is a danger that they will be spread to uninfested or only slightly infested areas. A satisfactory procedure with sugar beet, in all but exceptionally bad attacks, is to send both infected and healthy roots to the factory for processing.

There are persistent reports in the literature of the occurrence of the disease in severe form in wet places in fields, whereas Exp. 3 shows that excessive moisture tends to depress mycelial development. The probable explanation is that the wet conditions favour the survival of the sclerotia, as a result of reduced aeration, microbiological activity and nitrogen supply. In addition, the weed population is normally greater and more persistent in wet places, and this helps to perpetuate the fungus if susceptible species are present. The amount of inoculum persisting in these wet places apparently outweighs the depressing effect of the reduced aeration on the mycelial development.

It is possible that control by the application of fungicides to the soil would be practical in valuable horticultural crops such as asparagus; Ogilvie &

Mulligan (1934) report some success with 1 : 1600 mercuric chloride solution. It is unlikely, however, that such treatments would be practical on a field scale.

The field incidence of severe infection on alkaline soils make the apparent application of the results of Exp. 3, in which incorporation of lime with the soil completely eliminated infection, of doubtful value. The lime application to the pots corresponded to a dressing of approximately 35 tons/acre of hydrated lime, and the attendant disturbances to fertility would make such treatment on a large scale impractical, especially as the chances are that the soil will aready be alkaline. Heavy liming of centres of severe infestation in a field warrants further investigation as a means of localized disinfection.

The application of fertilizers appears to be a more promising method of reducing infection. The reductions of 26 and 24 % in consecutive years on the same plots by applications of 90 lb./acre of nitrogen as sulphate of ammonia may be compared with the control of *Sclerotium rolfsii* on sugar beet advocated by Leach & Davey (1942), whose experiments showed a reduction in infection of 28 % for 50 lb. of nitrogen and 54 % for 100 lb. of nitrogen/acre. Dressings of organic manure have also been successfully used in the control of *Phymatotrichum omnivorum*, especially when used in conjunction with deep tillage (Mitchell *et al.* 1941), and there is some evidence from Exp. 2 that farmyard manure dressings will reduce infection with *Helicobasidium purpureum*.

The authors wish to express their indebtedness to Mr H. H. Stirrup, Midland Agricultural College, in whose department this work was undertaken for the Sugar Beet Research and Education Committee; to the agricultural and tarehouse staffs of the Bardney, Brigg and Kelham factories of the British Sugar Corporation Ltd. for their help in collecting diseased roots and the survey work; to Messrs W. Parker and Son, Temple Grange, Lincoln, for providing the facilities for Exp. 1; to Messrs D. J. Finney and M. H. Quenouille of Rothamsted Experimental Station for help with the statistical analyses; also to Mr S. D. Garrett, Rothamsted Experimental Station, for many helpful suggestions during the course of the investigation and for criticizing the manuscript.

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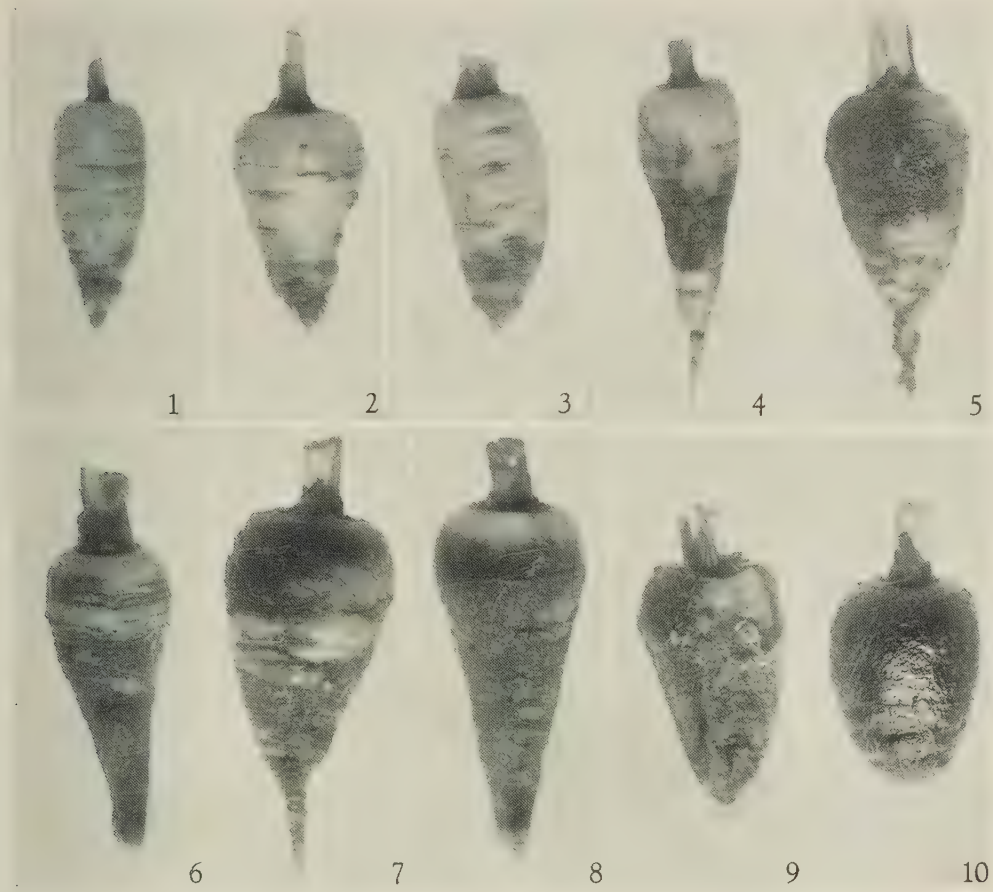

Fig. 1.

Fig. 2*a*.

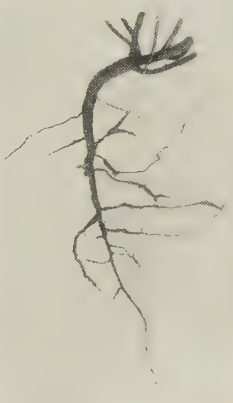

Fig. 2*b*.

EXPLANATION OF PLATE 12

Fig. 1. Series of carrots with varying degrees of violet root rot infection (1 = light, 10 = heavy) which were used as standards for grading experimental infections.

Fig. 2. Roots of *Senecio vulgaris* (*a*) and *Euphorbia Helioscopia* (*b*) on which sclerotia of *Helicobasidium purpureum* have been produced.

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The influence of potato variety on the cyst of the potato-root eelworm, *Heterodera rostochiensis* Wollenweber

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(With 12 Text-figures)

Experiments are described with eelworm cysts obtained from the roots of four potato varieties, Doon Star, Arran Banner, Redskin and Kerr's Pink. In a cyst-type: excretion-type hatching trial carried out in autumn, each group of cysts was stimulated by root excretions derived from the four types of plants. There were significant differences in the response to the root excretions from the different sources, and in the number of larvae emerging from the different cyst types, the number being highest for Arran Banner and practically nil for Doon Star cysts; but there was no correlation between cyst type and excretion type.

In experiments the following spring with the same cysts, emergence was heavy for all cyst types; but it began considerably later for Doon Star than for the other cyst types. The lack of emergence from Doon Star cysts in the previous autumn was, therefore, not due to the death of the contents of these cysts.

Fewer larvae emerged from Doon Star cysts than from either Arran Banner or Redskin (experiments with Kerr's Pink were discontinued). Doon Star cysts were smaller; however, comparing values for larval emergence and cyst volume for the different cyst types by the method of covariance, the larval emergence was found to be significantly lower for Doon Star cysts than for cysts of the other types, even after adjustment for cyst size.

The number of eggs in each cyst was determined. Analysis by the method of covariance showed that, after adjustment for cyst size, there were significantly fewer eggs in Doon Star cysts than in cysts of the other types.

A similar analysis was carried out of the relationship of larval emergence and egg number. The regression coefficients show that for each cyst type a constant proportion of eggs hatched; but the proportion is smaller in Doon Star cysts, for, after adjustment for egg number, the value for larval emergence is still significantly lower. Fewer larvae therefore emerged from Doon Star cysts partly because the cysts are smaller, partly because, for the same volume, they contain fewer eggs, and partly because a smaller proportion of these eggs hatched during the course of the experiments.

The results are discussed in relation to a recent paper by Gemmell (1943). While the differences may be due to resistance on the part of the host plant, as claimed by this worker, there are other possibilities; it is considered that these should be investigated before the hypothesis of 'resistance' is accepted. In particular, it is suggested that the differences in cyst size and egg number may be due to differences in the seasonal behaviour of the different potato varieties; differences in the hatching response of the cysts may then be due to the fact that the eelworm cyst responds to root excretions as a unit and that egg concentration is a factor affecting the nature of the unit.

Introduction

Gemmell (1943) has recently shown that the potato-root eelworm exhibits a remarkable response to the variety of the potato plant which it has parasitized. Working with four varieties of the domestic potato, *Solanum tuberosum*, he found that there were fewer and smaller cysts on Epicure and Doon Star than on Golden Wonder and Majestic; he claims moreover, that when stimulated by potato-root excretions, cysts from the first two forms produce fewer larvae than cysts of the same volume from the

other two forms. These results are of very great importance and there is little doubt that his paper has placed the study of this subject on a new footing. It is, however, difficult to agree with his conclusions, for all the differences reported are attributed to 'resistance' on the part of the host plants; the evidence for this seems to be inadequate.

The present work was begun in 1941, independently of Gemmell's and with the object of investigating, on the one hand, varietal resistance and, on the other, the response of eelworm cysts from different types of plants to root excretions derived from the same and different varieties. The latter object was of particular importance to work proceeding on eelworm resistance in South American species of potato plants (Ellenby, 1945). In the main, the present findings confirm and extend those of Gemmell (1943), but they are interpreted differently.

Experimental

Cysts

Ten tubers of each of ten varieties of the domestic potato were planted in infested soil in the spring of 1941; at the end of the season, the plants were lifted, and the eelworm cysts removed from the roots and stored in dry soil in paper bags. The cysts used in Gemmell's work were obtained by a different procedure; plants growing in sand were watered with suspensions of larvae derived from the same parent cysts so that each plant received about the same number of larvae. The cysts obtained from the roots of these plants were, therefore, probably more uniform than those used in the present work; nevertheless, the area from which the latter were derived was so small that it is doubtful whether there would be any marked difference due to the parentage of the cysts; and the behaviour of the cysts in each group was, in many respects, remarkably uniform.

Hatching trials 1943

Originally, it was intended to carry out hatching trials in 1942; owing to pressure of other work this was impossible and the first hatching experiments were carried out in the autumn of 1943 when the cysts were 2 years old. Gemmell's paper (1943) had already appeared, and this affected the experimental procedure; it was decided to concentrate on four out of the ten types of cysts available, viz, those derived from the following varieties, Doon Star, Kerr's Pink, Redskin and Arran Banner; these are sometimes described, for convenience, as cyst types, Doon Star cysts, etc.

The object of this first experiment was to investigate the response of the different cyst types, (*a*) to root excretions from the same type of plant, and (*b*) to root excretions from different types of plants. Accordingly, four lots of fifty cysts derived from Doon Star plants were taken, and each lot was stimulated by root excretions derived from a different type of plant; at the same time, the same procedure was followed with the cysts derived from the other three types of plants. The whole experiment was therefore a 4×4 cyst-type: excretion-type trial involving 800 cysts. Root excretions were obtained by standing the whole root system of a plant of a particular variety in water for 2 hr. A single cyst technique (Ellenby, 1943) was used. Freshly prepared root excretions were supplied every 5 days, and larvae were counted and removed every 2 or 3 days until emergence had ceased.

Results

The experiment was begun at the end of August, and, as expected for this time of year, the number of larvae emerging was small in all groups; it was therefore not considered worth while to treat the individual cyst totals separately. The total emergence for each group of fifty is shown in Table 1.

Table 1. *Cyst-type: excretion-type trial. Total number of larvae emerging from* 50 *cysts*

	Source of root-excretions				
Source of cysts	Arran Banner	Doon Star	Redskin	Kerr's Pink	Means
Arran Banner	161	421	446	192	305
Doon Star	15	15	1	0	8
Kerr's Pink	79	120	212	44	114
Redskin	25	210	129	84	112
Means	70	191	197	80	

Standard error $= \pm 34{\cdot}93$

An analysis of variance showed that there were statistically significant differences due both to cyst type ($p < 0{\cdot}01$) and excretion type ($p = 0{\cdot}05$). With a Standard error for the difference between any two means of $\pm 49{\cdot}40$, cysts from Arran Banner plants are shown to produce significantly more larvae than any of the other types ($p = 0{\cdot}05$ for Kerr's Pink, $p < 0{\cdot}01$ for Redskin, and $p < 0{\cdot}001$ for Doon Star). The differences produced as a result of the treatments with the different samples of root excretions are not so great; the mean values for Redskin and Doon Star are highest and almost equal, and those for Arran Banner and Kerr's Pink also almost equal and lowest. The difference between either member of the high group and either member of the low group is significant in three of the four cases ($p = 0{\cdot}05$) and just falls below the level of significance in the case of the difference between Doon Star and Kerr's Pink.

Discussion

Triffitt (1930) showed that the quantity of root excretions given off by four potato seedlings into 5 c.c. of water was sufficient to induce maximum emergence of larvae of the potato-root eelworm, and that

further increase in the strength of the solutions was without effect. The solutions used in the present series of experiments were considered to be far stronger than this, and it is therefore of considerable interest that the solutions from the different varieties should have produced such different effects; moreover, fresh solutions were prepared every 5 days so that during the course of the experiment a number of different plants of each type would have been used. The question of the strength of the solution of root excretion is such a difficult one, however, that it would be unwise to draw any definite conclusions concerning varietal differences from these experiments; but clearly it will be interesting to investigate the matter further, using a recently developed technique (Ellenby, 1944*a*) which does at least provide some basis for comparing the strength of solutions of root excretions.

Although these differences in response to the various types of root excretions were shown, Table 1 shows that there is no correlation between cyst type and excretion type; the most important variable is clearly the source of the solutions used, larval emergence being high in certain solutions, and low in others, irrespective of the type of cyst. These results, however, were obtained with strong solutions of root excretion, and it does not necessarily follow that they would be of the same type if weaker solutions were used.

The most surprising result, as far as cyst type is concerned, is undoubtedly the response, or rather the lack of response, shown by the cysts obtained from Doon Star plants; only thirty-one larvae emerged from the 200 cysts of this type, compared with 448 from Redskin, 455 from Kerr's Pink and 1220 from Arran Banner. In actual fact, these thirty-one larvae came from nine of the 200 cysts, the vast majority thus producing no larvae at all; all types of cysts had been stored under the same conditions, but the possibility that the Doon Star cysts had not stored as well as the cysts from the other types of plants could not be excluded. The cysts were therefore kept until the following year when they were used in the experiments described below.

Hatching experiments 1944

The experiment of 1943 was continued in April 1944 with the same cysts; they had been stored dry during the winter at room temperature in the petri dishes in which the autumn experiment had been carried out, each cyst occupying its original position. A single variety of plant was used as a source of root excretion, in view of the results with the root excretions from the different varieties, and it was prepared in the usual way. Originally, it was intended to use the whole 800 cysts; but the emergence of larvae was so heavy that the experiment became unmanageable and after it had proceeded for 2 weeks its scope had to be reduced considerably. Thereafter the hatching experiment was confined to one lot of fifty cysts from Arran Banner plants, one lot of fifty from Redskin plants, and two lots of fifty Doon Star cysts. Emergence of larvae had practically ceased by the beginning of June, and the experiment was then discontinued.

Results.

In the autumn of 1943, only nine out of the 200 Doon Star cysts produced larvae, and they only a total of thirty-one; the experiments in the spring of 1944 showed conclusively that this lack of response was not due to any lack of viability of these cysts, for larvae emerged from most of them, the average number emerging per cyst being about seventy. But it was very noticeable that emergence from Doon Star cysts began later than that from the others.

Fig. 1 shows the logarithm of the total larval emergence at any time; while emergence in the Redskin and Arran Banner cysts began after 7 days, in Doon Star it did not begin until about 4 days later. It is difficult to demonstrate this difference graphically as 4 days is only a small part of the experimental

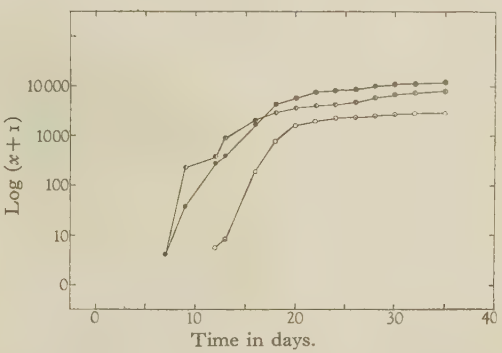


Fig. 1. Larval emergence from cysts from three varieties of potato plant; semi-logarithmic plot. Redskin ●, Arran Banner ◐, Doon Star ○. Emergence begins later in the Doon Star cysts.

period and because the number of larvae emerging during the first fortnight is very small compared with the total emergence; the difference, which may be of some importance, is emphasised by the logarithmic plot. All the 800 cysts of the autumn experiments were being used at the time, although the diagram is based on the data obtained from only the limited number of cysts; the Doon Star cysts differed in this way from all the others including Kerr's Pink.

Apart from this difference in the rate at which the cysts responded to root excretions, the groups of cysts also differed markedly in their productivity: while each Doon Star cyst produced an average of

seventy-one larvae, the value for Redskin is 253, and for Arran Banner 168; but the variation in total number of larvae produced per cyst is considerable in all groups. In treating data of this type, giving a much skewed distribution, a logarithmic transformation is used, already discussed in relation to a previous investigation (Ellenby, 1944*b*); the values of $\log(x+1)$ are used in the analysis, where x is the number of larvae per cyst, instead of $\log x$, so as to avoid difficulties with cysts producing no larvae. The results of an analysis of variance of total larvae, per cyst, using this transformation, are shown in Table 2.

TABLE 2. *Emergence of larvae from cysts from three potato varieties. Mean values of* $\log(x+1)$ *and* S.E. *where* x *= total number of larvae emerging, per cyst.*

Cyst type ...	Arran Banner	Redskin	Doon Star
Mean values of $\log(x+1)$ and S.E.	2·109 ±0·097	2·246 ±0·096	1·336 ±0·070

The mean value of $\log(x+1)$ for Doon Star, 1·336, is very much less than the value for either of the other two forms. The difference between Redskin and Arran Banner is not significant ($p>0{\cdot}1$), but with S.E.'s derived from the error mean square of ±0·119 and ±0·120 respectively, the differences between either of these forms and Doon Star is very highly significant ($p<0{\cdot}001$). But this takes no account of differences in the volume of the cysts in the various classes, and it is well known that the number of eggs varies with the size of the cyst (Triffitt, 1929, O'Brien & Prentice, 1931).

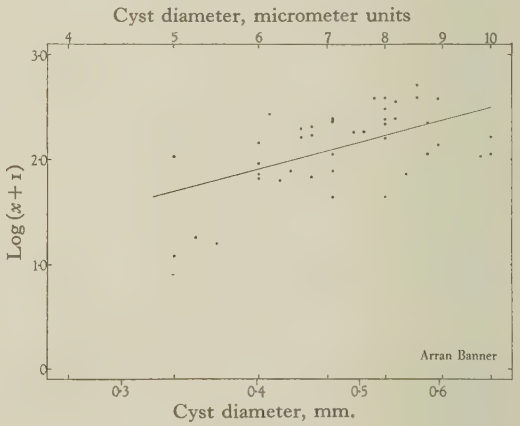
Cyst volume and larval emergence

Gemmell (1943), as already mentioned, found that Doon Star and Epicure cysts produced fewer larvae than cysts formed on two other varieties of plants, varieties other than Redskin and Arran Banner. In order to consider the larval emergence independently of the size of the cysts, he divided the cysts into five arbitrary groups of different cyst volume, and then made comparisons between the members of the different classes. This procedure, though useful in a preliminary analysis, does not use the data in the best way; in fact, no test was made of the significance of the differences between members of the same size classes. The matter is better tested by an analysis of co-variance which takes account of the variation in the data at any size level.

The dimensions of the cysts were obtained with an eye-piece micrometer used in one ocular of a binocular dissecting microscope; the accuracy of measurement was about ±3%. The length to the base of the neck and the width were measured, and the means of the two values used in the estimation of cyst volume; in practice, the two values usually agreed and it was found that only the width need be measured except in cysts which departed from the apparently spherical. Calculations were carried out in micrometer units to save the labour of conversion; in view of the comparative nature of the present work, this should constitute no difficulty. Ten micrometer units ≡ 0·66 mm. In the text, values for diameter are given in italics when in micrometer units; thus, mean diameter = *7·5*, or log *0·88*, or 0·50 mm.: however, in all diagrams, values for diameter are given in both micrometer units and mm.

Results

In Figs. 2–4, the values of $\log(x+1)$ have been plotted against the logarithm of the diameter for the three cyst types. Essentially, the plot is therefore that of larval emergence and cyst size on a double



Figs. 2–4. Larval emergence and cyst size for cysts from three varieties of potato plant. Values of $\log(x+1)$ are plotted against the log of the diameter.

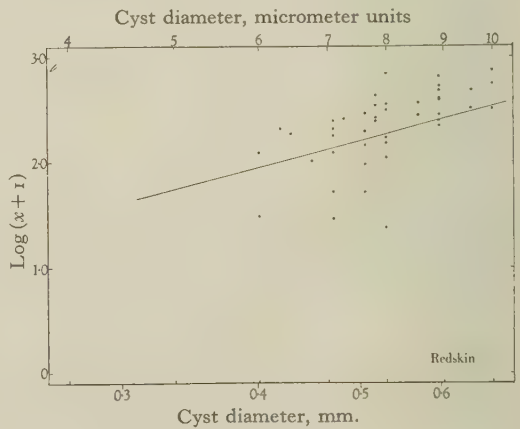


Fig. 3.

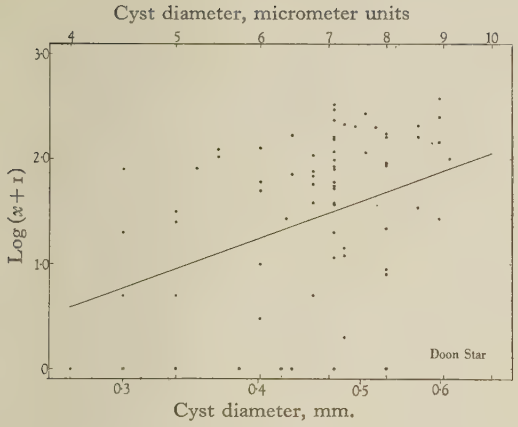


Fig. 4.

logarithmic grid; taking the log of the diameter saves the conversion to volume, but nevertheless enables the relationship of larval emergence to cyst volume to be determined.

The variation in the data is considerable for all cyst types, with correlation coefficients of +0·55, +0·31 and +0·36, for Arran Banner, Redskin and Doon Star respectively; but all coefficients are statistically significant ($p<0{\cdot}01$, $<0{\cdot}05$ and $<0{\cdot}01$ respectively). In order to compare the values of log $(x+1)$ independently of cyst size, the method of co-variance is used. For this purpose the regression coefficients of log $(x+1)$ on log diameter for the three sets of data are first calculated. These values, together with the above correlation coefficients, are shown in Table 3; as might be expected they are all in the region of 3, showing that the larval emergence is roughly proportional to the volume.

The high value of +3·7 for Doon Star may be in agreement with the findings of Gemmell (1943) that the largest cysts from this variety did not differ in productivity from similar cysts of other sorts; but the variation is so large that it is difficult to attach any significance to it; in fact, with a standard error of regression of ±0·875, the coefficient does not differ significantly from 3. The mean square for error, 0·3704, is considerably greater than the mean square for differences among lot regressions, 0·0906, showing that there are no significant differences between the three regression coefficients. The error regression coefficient is, therefore, then calculated for all the data; this enables the values of log $(x+1)$ at the experiment mean value for log diameter to be estimated, i.e., the emergence for a cyst of each type, at a diameter equal to the mean of the cysts of all the types. These 'adjusted mean' values, together with their standard errors, are tabulated in Table 4, There is little difference between the values for Redskin and Arran Banner, but with an adjusted mean value of 1·5134, Doon Star differs significantly from either of the other two forms ($p<0{\cdot}001$). However, there are significant differences in the mean values for log diameter for the three cyst types, so that it is necessary to interpret the results of the analysis of co-variance rather carefully; but, clearly, the conclusion is justified that fewer larvae emerge from Doon Star cysts than from Arran Banner or Redskin and that the differences are greater than can be explained by the differences in cyst size.

TABLE 3.* *Larval emergence and size of cyst. Correlation and regression coefficients for values of log $(x+1)$ and log cyst diameter, for cysts from three potato varieties, where x = total number of larvae emerging per cyst.*

Cyst type	Correlation coefficient	Mean value log $(x+1)$	Mean value log diameter	Regression coefficient	S.E. of regression
Arran Banner	+0·55	2·1228	**0·8616**	+2·7027	±0·6467
Redskin	+0·31	2·2600	**0·8996**	+2·7047	±1·2385
Doon Star	+0·36	1·4214	**0·8284**	+3·7048	±0·8752

* There are slight differences in the values for log $(x+1)$ in this table and Table 2. Cysts are sometimes wrinkled so that it is not always possible to measure them accurately; there are therefore differences in the items dealt with in the two analyses. Later in the paper, when egg number is considered, slight differences will again be found in the values for apparently identical items; this is due, partly to an occasional technical mishap in the determination of egg number, and partly to the inclusion of fifty additional Arran Banner cysts which were not used in the spring hatching experiments and for which, therefore, no data on larval emergence were available. The principle has been to use all available data and not to exclude from an analysis values, for example, for larval emergence for a particular cyst, because, for a subsequent analysis, it was impossible to obtain an accurate estimate of its dimensions.

The mean values for log $(x+1)$ are essentially the logs of the geometric means for larval emergence (Ellenby 1944*b*): the geometric means corresponding to the adjusted mean values of log $(x+1)$ of Table 4 are, for Arran Banner, Redskin and Doon Star, 127·9 132·0 and 32·6 respectively; the differences are therefore large even though the comparison of geometric means reduces their magnitude.

Egg number and cyst volume

Gemmell (1943) states that cysts from the 'resistant' varieties show no 'notable' reduction in

egg content compared with the susceptible varieties; he considers that a large number of the eggs in the former cysts are incapable of hatching. Unfortunately, no figures are presented to support these statements; in any case, it seemed desirable to investigate the relationship between egg number and cyst size for the cysts used in the present work. Accordingly the walls of the cysts were dissolved in dilute sodium hypochlorite solution (Smedley 1936) and the number of full eggs remaining in each cyst recorded; no attention was paid to the state of contents of the eggs as they may have been affected by the hypochlorite treatment. An additional fifty Arran Banner cysts were included in these determinations. Using the values for larval emergence from the two series of hatching experiments, the total for the number of eggs originally in each cyst were thus obtained.

Results

Values for total eggs are plotted against cyst volume in Figs. 5, 6 and 7. The variation in the number of eggs per cyst is considerable at all levels

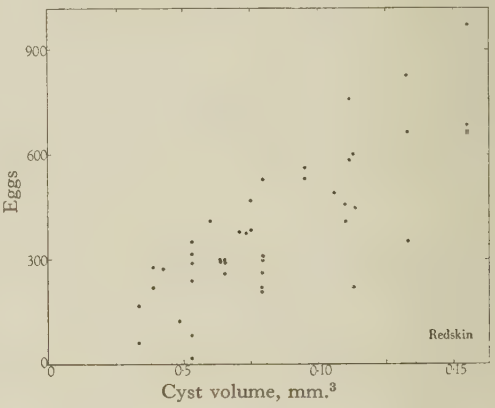


Fig. 6.

Prentice (1931) which is composed of six points all of which lie almost exactly on a straight line.

In order to decide whether there were any significant differences in the number of eggs per cyst at any size level, for the different cyst types, a similar procedure was adopted to that used in the analysis of the values of log $(x+1)$ in relation to cyst size. The values of log E and log D were determined, where

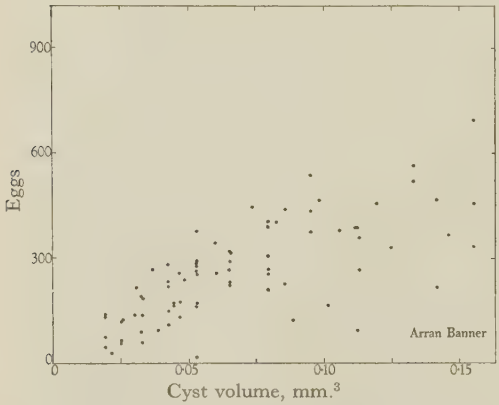
TABLE 4. *Analysis of co-variance. Larval emergence from cysts of similar size. Comparison of adjusted mean values of log* $(x+1)$ *for cysts of three potato varieties, at log cyst diameter* **0·8567**, *where* x = *total number of larvae emerging per cyst.*

Error regression coefficient (b) = +3·2519

Mean value of log cyst diameter = **0·8567**

Cyst type	Mean value log diameter	Deviation for experiment mean (p)	Product bp	Mean value log $(x+1)=q$	Adjusted mean $(q-bp)$	S.E. of estimate*
Arran Banner	**0·8616**	**+0·0049**	+0·0159	2·1228	2·1069	±0·0927
Redskin	**0·8996**	**+0·0429**	+0·1395	2·2600	2·1205	±0·0896
Doon Star	**0·8284**	**−0·0283**	−0·0920	1·4214	1·5134	±0·0692

* Based on error mean square.



Figs. 5–7. Egg number and cyst volume for cysts from three varieties of potato plant.

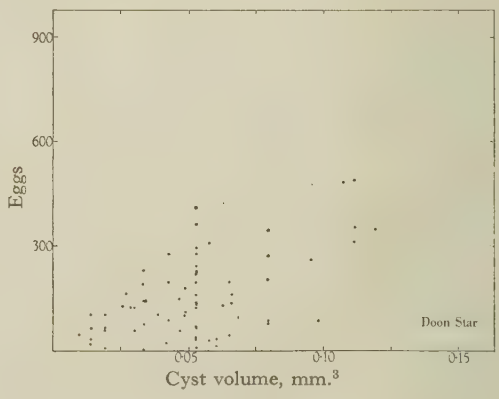


Fig. 7.

of cyst size; clearly, cyst size is not the only factor determining egg number. In this respect the diagrams differ markedly from that of O'Brien &

E = total number of eggs in a cyst of diameter D micrometer units, i.e. larvae emerged + unhatched eggs remaining. These values are plotted in Figs. 8, 9 and 10. The calculated linear regression lines fit the data fairly closely for both Arran Banner and Redskin (Figs. 8 and 9), but there is much more scatter in the case of Doon Star (Fig. 10); in fact, the correlation coefficients for the first two are +0·300 and +0·315 respectively, and only +0·165 for Doon Star.

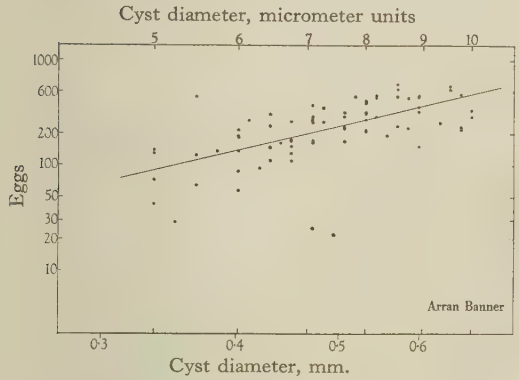
The first two values are low, but not unexpectedly low for data of this sort; on the other hand, the value for Doon Star, +0·165, is very low indeed and shows that the egg content of these cysts varies much more than that of cysts of the other types.

The analysis of covariance was carried out as in the case of the analysis of larval emergence and cyst volume. The regression coefficients, together with their S.E.'s calculated for each set of data separately, are shown in Table 5. As might be expected, they are all round about +3·0, showing that the number of eggs is roughly proportional to the cube of the diameter. It is interesting that the value for Redskin, +3·59, is so much higher than +3·0, while that for Arran Banner, +2·34, is so much lower. The S.E. of regression, calculated from the Redskin data alone, is ±0·196, so that the value of +3·59 differs significantly from +3·0; there is therefore a significant tendency for the number of eggs per unit volume to increase with the volume of the cyst. On the other hand, with a S.E. of ±0·353, the value for Arran Banner, +2·36, does not differ significantly from +3·0 although it approaches the level of significance (p between 0·1 and 0·05). Clearly, the possibility of the number of eggs per unit volume increasing or decreasing in different cyst types must be borne in mind.

These tests were carried out on the individual sets of data. If the data are considered as a whole, the analysis of co-variance shows that there are no differences between the regression coefficients, the mean square for deviations from individual lot regressions, 0·106, being almost equal to the mean square for differences among the individual re-

TABLE 5. *Egg number and cyst size. Correlation and regression coefficients for values of log E and log cyst diameter for cysts from three potato varieties, where E = number of eggs per cyst before emergence of larvae.*

Cyst type	Correlation coefficient	Mean value log E	Mean value log diameter	Regression coefficient	S.E. of regression
Arran Banner	+0·300	2·3429	0·8658	+2·3429	±0·353
Redskin	+0·315	2·5120	0·8984	+3·5901	±0·196
Doon Star	+0·165	2·0743	0·8284	+2·6338	±0·613



Figs. 8–10. Egg number and cyst volume for cysts from three varieties of potato plant. Values for egg number are plotted against cyst diameter on a double logarithmic grid.

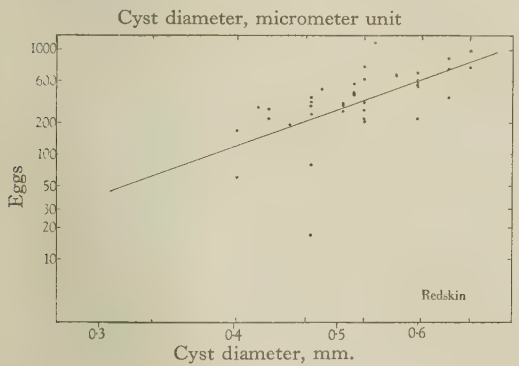


Fig. 9.

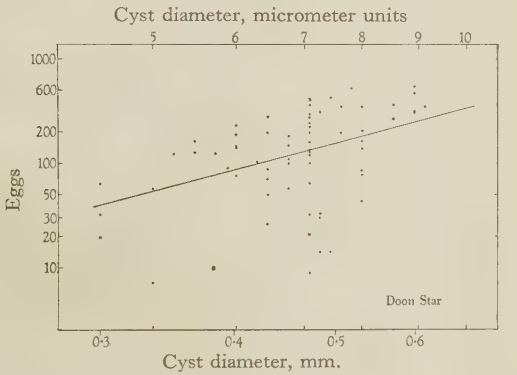


Fig. 10.

gressions, 0·088. The variation in the Doon Star data is so large, however, that it was considered worth while to carry out a separate analysis for Redskin and Arran Banner. The difference in the regression coefficients for these two forms almost reaches the level of significance. The mean square for the difference between lot regressions is 0·198, while the mean square for error is 0·063; this gives a value for F of 2·82, which is just below the 5% level.

Although there are no clear differences in the slopes of the curves, there are distinct differences in their levels. The mean square for error, 0·105, is very much smaller than the mean square appropriate to this test, 1·019, giving a value for F of 9·66, which is a very significant value indeed. The values for adjusted means are shown in Table 6. With a S.E. of ±0·061, the difference between Arran Banner and Redskin is not significant ($p=0·2$); on the other hand, the difference between either of these types and Doon Star is highly significant (p almost 0·001), showing that the number of eggs per cyst in Doon Star is significantly smaller than in the other types, and that this difference is greater than can be explained by difference in cyst size.

The agreement in the proportion of Arran Banner and Redskin eggs which hatched is remarkable, 67·7% in both cases; as the numbers involved are so very large, this agreement is probably a true representation of the facts and not a coincidence. On the other hand, the value for Doon Star is 42·0%, showing that less than half the eggs hatched. This difference in percentage hatch is of considerable interest and one which deserves a more detailed study than is provided by the above method in which all the eggs are 'pooled'; it seemed worth while to carry out a test which considered hatching, not on the basis of 'pooled' eggs, but in relation to the eggs in each cyst. Accordingly, an analysis of co-variance of larvae emerged, and total eggs per cyst, was carried out using the values for $\log(x+1)$ and $\log E$.

The values for the regression coefficients are +1·2804, +1·0024 and +0·8814, for Arran Banner, Redskin and Doon Star respectively; the mean square for deviations from individual lot regressions, 0·2750, is almost equal to the mean square among lot regressions, 0·2742, showing that there are no differences in the values for the regression coefficients. The closeness of all the value to +1·0 is noteworthy, indicating, as the values considered are logs, that for any cyst type a constant proportion of eggs hatch irrespective of the number of eggs contained in the cyst.

Although the regression coefficients are similar, there are marked differences in the relationships of the mean values for $\log(x+1)$ and $\log E$ for the three cysts types, and the analysis of co-variance might be expected to support the argument that there are differences in the proportion of eggs which hatch. In Table 8 the results of this analysis have been set out in a comparison of adjusted mean values. At the experiment mean value for $\log E$ of 2·2121, the values of $\log(x+1)$ for Arran Banner, Redskin and Doon Star, respectively, are 1·9779, 2·0139 and 1·5248; the differences between Arran Banner or Redskin and Doon Star are very highly significant ($p<·001$). However, once again there are statistically significant differences between the mean values for the independent variate, in this case $\log E$. Nevertheless it is

TABLE 6. *Analysis of co-variance. Egg number in cysts of similar volume. Comparison of adjusted mean values of log E for cysts from three potato varieties, at log cyst diameter* **0·8592** *where E = total number of eggs per cyst.*

Error regression coefficient (b) = +2·6342

Mean value of log cyst diameter = **0·8592**

Cyst type	Mean value log diameter	Deviation from experiment mean (p)	Product bp	Mean value log E (q)	Adjusted mean ($q-bp$)	S.E. of estimate based on error mean square
Arran Banner	**0·8658**	+0·0066	+0·0174	2·3429	2·3255	±0·0368
Redskin	**0·8984**	+0·0392	+0·1033	2·5120	2·4087	±0·0484
Doon Star	**0·8284**	−0·0308	−0·0811	2·0743	2·1554	±0·0377

Emergence of larvae in relation to number of eggs

Doon Star cysts produce fewer larvae per cyst at all levels of cyst size; they also contain fewer eggs. Is the difference in the number of eggs per cyst sufficient to explain the difference in larval emergence? A simple test can be made by comparing the proportions of eggs contained in the cysts which emerged during the hatching experiments. These results are shown in Table 7.

TABLE 7. *Emergence of larvae in relation to number of eggs in cysts from three potato varieties.*

Cyst type	Eggs hatched (= larvae produced)	Eggs remaining	Hatch %
Arran Banner	8095	3867	67·7
Redskin	12295	5853	67·7
Doon Star	6498	8913	42·0

true to say that after adjustments for differences in egg number there are still significant differences in larval emergence. Clearly, fewer larvae emerged from Doon Star cysts partly because the cysts are smaller, partly because, for the same volume, they contain fewer eggs, but also because a smaller proportion of these eggs hatched during the course of the experiments.

Larval emergence in relation to cyst size and to egg number

Earlier on it was noted that the regression coefficient of $\log(x+1)$ and log diameter was $+3{\cdot}7$ in Doon Star, suggesting that the larval emergence may alter with the size of the cyst. The variation in the Doon Star data is very large, however, and the coefficient does not differ significantly from $+3{\cdot}0$. The point is of some interest and it seemed worth while to examine it in more detail. Is there any evidence that the proportion of eggs which hatch varies with the size of the cyst? Clearly this question can be considered independently of cyst type.

A serious difficulty in the interpretation of results on emergence of larvae from eelworm cysts is the very great variation in the data; some cysts produce several hundred larvae, while others produce very few or even none at all. As amply shown in this and other work, not only do the cysts in an experimental group vary in volume, and therefore in the number of eggs they contain, but even cysts of the same volume may contain very different numbers of eggs; finally, the response of the groups of eggs may be different. It is possible to get some idea as to the relative importance of these factors by considering, with figures for larval emergence, the values plotted in Figs. 11 and 12 for Arran Banner as values of two constants which show no trend with increasing

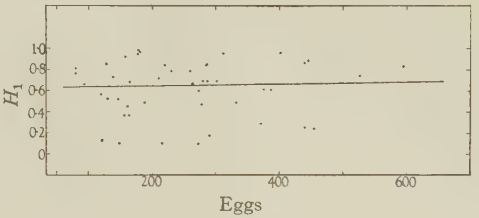


Fig. 11. Hatching as a function of egg number for cysts from Arran Banner plants. Values for

$$\frac{\text{larval emergence}}{\text{egg number}} = H_1$$

are plotted against total eggs for each cyst.

TABLE 8. *Analysis of co-variance. Larval emergence and egg number. Comparison of adjusted mean values of* $\log(x+1)$ *for cysts from three potato varieties at* $\log E = 2{\cdot}2131$, *where* x = *number of larvae emerging from a cyst containing* E *eggs.*

Error regression coefficient (b) = $+0.9464$

Mean value of $\log E = 2{\cdot}2131$

Cyst type	Mean value $\log E$	Deviation from experiment mean (p)	Product bp	Mean value $\log(x+1)=q$	Adjusted mean ($q-bp$)	S.E. of estimate*
Arran Banner	2·305	+0·092	+0·0871	2·065	1·9779	+0·0758
Redskin	2·491	+0·278	+0·2631	2·277	2·0139	±0·0782
Doon Star	2·023	−0·190	−0·1798	1·345	1·5248	±0·0556

* Based on error mean square.

The most suitable series of data is that derived from the Arran Banner cysts, for of the three types they cover the most extensive range of cyst size; Redskin cysts are mainly large; Doon Star mainly small cysts. In Fig. 11 the values for

$$\frac{\text{larval emergence}}{\text{egg number}} = H_1$$

are plotted against values of E = total eggs for each cyst; similarly, in Fig. 12, values for

$$\frac{\text{larval emergence}}{D^3} = H_2,$$

where D = cyst diameter in micrometer units, are plotted against cyst volume; values for H_2 happen to be about a tenth of the values for H_1. Clearly the lines fitting both these series of data are horizontal, showing that the values for neither ratio change with increasing cyst size. The regression coefficients are $+0{\cdot}00102$ and $-0{\cdot}0105$ respectively, and neither of these differs significantly from zero; the 'hatching rate' does not vary with the size of the cyst, or with the number of eggs included.

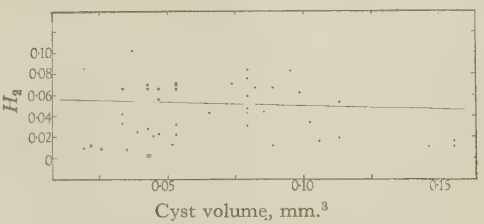


Fig. 12. Hatching as a function of cyst size for cysts from Arran Banner plants. Values for $\frac{\text{larval emergence}}{(\text{cyst diameter})^3} = H_2$ are plotted against cyst volume.

cyst volume or egg number, and which therefore show only random variation at any level of cyst size or egg number.

Mean values are shown in Table 9, together with their standard deviations; the relationship between the S.D. and the mean value is expressed as a percentage, and, for comparison, corresponding mean values etc., for larval emergence, in natural numbers. Expressing the emergence per unit of cyst volume improves the data appreciably compared with the natural numbers, but the values for the ratio of larval emergence and total eggs show slightly greater variation. It is probable, however, that the reverse would be the case when dealing with a heterogeneous population of cysts from the field; the variations in egg number might be so large that there would be a real gain in expressing emergence in relation to egg number. Clearly, although factors of cyst size and egg number are important variables affecting eelworm data, biological variation in the material is of decisive importance.

TABLE 9. *Arran Banner cysts. Mean values for larval emergence* (x), $x/E = H_1$, $x/D^3 = H_2$, *where* E = *total eggs contained in a cyst,* D *micrometer units diameter, from which* x *larvae have emerged.*

	Mean value	S.D.	S.D. / mean value × 100
Larval emergence (x)	168·81	±116·10	69·0
$x/E = H_1$	0·5209	±0·375	72·0
$x/D^3 = H_2$	0·4527	±0·2565	56·5

Discussion

The results show that the Doon Star cysts examined in this work differed from Redskin and Arran Banner cysts in the following ways: (1) practically no larvae emerged in August and September; (2) in May and June emergence began considerably later, i.e. the hatching response is different; (3) the average cyst volume was less; (4) fewer larvae emerged per cyst, and the difference is not due to cyst size, for it is significant after the data are adjusted for cyst size; (5) the cysts contained fewer eggs per unit of volume; (6) there was greater variation in the number of eggs per cyst; (7) fewer of the contained eggs hatched during the course of an experiment, i.e. the difference in the number of larvae which emerged was greater than the difference in the number of eggs. Experiments also showed that the response to potato root excretions of these three cyst types and also of Kerr's Pink cysts was no different whether the stimulation was carried out with root excretion derived from the same, or a different potato variety, at any rate when strong solutions are used; on the other hand, these experiments suggested that there may be differences in the quantity of root excretions produced by different varieties of potato plant. There is also evidence that in Redskin cysts the number of eggs per unit of cyst volume increases with the size of the cyst.

Gemmell (1943) has already shown that Epicure and Doon Star cysts are smaller than Golden Wonder and Majestic cysts, and that they also produce fewer larvae. To consider whether these differences are due to the size of the cyst, he groups the cysts in size categories and finds that, except in cysts of the largest size, the number of larvae which emerge is smaller even when the comparison is made between cysts belonging to the same size category; there is no difference between the cysts belonging to the largest size group. I have already pointed out that although this procedure is useful in a first comparison, it can hardly be regarded as sufficient for establishing the difference claimed; the size groups are arbitrary, and the variation in the data cannot be utilized efficiently; in fact no statistical test was carried out to determine whether the differences between the different cyst types belonging to the same size category are of statistical significance. In the present work, the data were examined by the method of co-variance, which is not open to these objections; the results show conclusively that Doon Star cysts produce fewer larvae than cysts of the same size belonging to the other two types examined in the present work. Moreover, the number of eggs contained in Doon Star cysts is very much less than the number contained in cysts of the same size belonging to the other types. Nevertheless, the difference in the emergence is greater than the difference in the number of eggs; while almost 70% of Redskin and Arran Banner eggs hatched during the experiment, the value for Doon Star was only about 45%.

Gemmell (1943) states that there was no 'notable' difference in egg numbers, a point which this work fails to confirm; this will be returned to later. The existence of differences in number of eggs per unit of cyst volume does not appear to be connected with differences in egg size. While the egg volume is not always constant, measurements of about 100 eggs showed no correlation either with cyst volume or with cyst type; this is in agreement with previous measurements by O'Brien & Prentice (1931), although Bessey (1911) considers that in *H. marioni*, small females tend to lay small eggs.

It has been suggested by Boyd (1943) that the density of the surrounding soil is the primary cause of the reduction in the size of the cyst on heavy soils; not only is the pressure of the soil on the young cyst thought to limit its size, but this in turn is considered to result in the formation of fewer eggs. It is very doubtful whether these suggestions can be supported. The present investigation has demonstrated that cysts are not as full of eggs as they might be; indeed, in most Doon Star cysts, there is room for many more eggs. Moreover, Gemmell (1943) frequently found

cysts of normal size which contained no eggs at all. It is unlikely, therefore, that the number of eggs is determined by cyst volume in any direct fashion, and this is supported by considerations of the shape of the egg. If the physical limitations of the cyst volume influenced the number of eggs by the pressure of its limiting walls, one might expect to find some evidence of pressure on the shape of the eggs. The packing together of cells has been extensively treated by Thompson (1942); cells may begin as spheres, but when packed together most economically, their shape differs very markedly from the spherical. The ellipsoidal shape of the egg of the eelworm provides no evidence of any packing effects of this sort; indeed, on theoretical grounds one would not expect the whole of the available space in the cyst to be occupied, for if the eggs were packed as tightly as possible, there would be no space available for hatched larvae. Nevertheless, the packing of the eggs is very close and there is very little 'free space'. For example, a cyst of 0·67 mm. diameter has a volume of $1{\cdot}57 \times 10^8 \mu^3$, while the average volume of the eggs examined was $9{\cdot}37 \times 10^4 \mu^3$. If the entire space inside the cyst were filled with eggs, that is, if there were no space between the eggs, the maximum number of eggs would be 1676; in fact, the maximum number found in a cyst of this volume was 969, showing that the 'porosity', or percentage of 'voids' (Dalavalle, 1943), was 40·3 % in this cyst. While most Doon Star cysts had fewer eggs per unit of cyst volume, the cyst having the highest number of eggs per unit volume was of this type with a diameter of 0·467 mm. and containing 413 eggs; the porosity in this case was only 27 %.

The closest packing of spheres (rhombohedral) gives a porosity of 25·95 %, while spheres in most open packing (cubical) have a porosity of 47·64 %. The best value obtained by Fraser (1935) in a study of a number of substances was given by marine sand; when 'compacted' this had a porosity of 34·78 % when dry and 38·52 % when wet. The best packing found in these eelworm cysts is therefore considerably better than these best values and is very near to 'tighest packing'; the fact that the particles studied were about 0·5 mm. diameter and therefore considerably larger than eelworm eggs, does not detract from this remarkable fact for the closeness of packing of particles of uniform shape is dictated by shape alone, and the different sizes of the sand particles should enable them to pack more closely than particles of uniform size.

Experiments with punctured cysts have demonstrated that the cyst must be regarded as a unit rather than as a mere collection of eggs in a protective covering (Ellenby, 1946), for more larvae emerge from punctured cysts than from controls; clearly, the response of the eggs contained inside a cyst is governed, to some extent at least, by the fact that the eggs are contained in a more or less closed system. The present investigation has shown that the response from Doon Star cysts is different from the response from other types; their seasonal response is different, they take longer to start producing larvae, and they produce fewer larvae. Although the eggs in some Doon Star cysts are as tightly packed as other types of cysts, on the whole, they contain far fewer eggs. If it is correct to consider that the cyst responds as a unit, it is possible that the differences in the response of Doon Star cysts are due to the fact that, containing fewer eggs, they constitute a different type of hatching unit. I have suggested elsewhere (Ellenby, 1946) that the cessation of hatching may be due to the accumulation within the cyst of an inhibitor, and that puncturing the cyst wall affects the time taken for the inhibitor to reach an effective concentration; it is possible that the differences in response of the cyst types examined may be bound up with similar diffusion problems related to the different types of cyst units. But more information is required before anything can be said on this point with confidence.

Gemmell (1943) considers that Doon Star and Epicure plants show 'resistance' as compared with Golden Wonder and Majestic. It is certainly true that the variety of potato plant affects the cysts borne on its roots, and that, in some cases, this would hinder the 'establishment of the nematode' or limit 'its potential reproductive capacity'. But is it correct to conclude that the potato variety is therefore exhibiting resistance? For example, if it were shown that these differences were due to the time at which the plants reached maturity, 'resistance' would not be attributed to the plant variety, even though the reaction of the plant 'hinders the establishment of the nematode'. Gemmell is aware of this point; 'Doon Star', he writes, 'reaches maturity at about the same time as Majestic', so that the larger number of 'aborted cysts in this case must be a varietal effect having the nature of true physiological resistance, and not the result of the time available for the nematode to complete its life cycle'. This point will be dealt with later; at the moment it will suffice to draw attention to the fact that hindering the establishment of the nematode is not *necessarily* a criterion of resistance in the strict sense of the term.

Barrons (1939), in a study of resistance to the root-knot eelworm, showed that larvae invaded the tissues of resistant plants but that they subsequently died, owing, in his opinion, to the production of a substance which neutralized the factor in the salivary secretions which stimulated the formation of the giant cells in the plant; he considered that they died of starvation. No extensive investigation of the nematode within the host was made either by Gemmell (1943) or myself, although examination of roots shows that the larvae have no difficulty in entering. Gemmell, however, considers that the smaller number of cysts obtained from the roots of Epicure and Doon Star

when they are subjected to the same degree of infection as the two other forms may be evidence that they are resistant. Clearly, as a result of these differences in cyst number, there would be considerable differences in the rates at which the disease would increase in the different cases; but is it correct to attribute this to 'resistance' on the part of the host plants? Gemmell argues that, possibly, the extent of root development was greater in the forms with more cysts 'increasing the chances of infestation, but there is equally the possibility that Epicure exhibited resistance. . . and a number of larvae either could not establish themselves or died prematurely. If one assumes this latter view to be correct, then Epicure and Doon Star may be said to exhibit true resistance to nematode infestation.' Consideration from his data for cyst number suggests, however, that it is far more likely that it is due to factors such as extent of root development.

Each plant, in Gemmell's work, received 20,000 larvae; from his figures for cyst counts in 50 g. of sand, the total number of cysts formed on each plant can be calculated; these are, for Epicure, Doon Star, Majestic and Golden Wonder, 470, 580, 880 and 1140 respectively. Strubell (1888) found equal numbers of males and females in the beet eelworm, though Moltz (1920) has shown that under certain conditions this may be altered. Assuming a 50:50 sex ratio, these figures mean that the numbers of female larvae which do not reach maturity are 9528, 9420, 9120 and 8886 respectively. (These figures, incidentally, do not indicate that the cultural conditions were bad; on the contrary, Triffitt (1930) in pot experiments obtained only 110 cysts from plants grown in pots inoculated with 1200 cysts.) Even in Golden Wonder, therefore, the best case, the loss of larvae is very close to 90%. Moreover, an entirely different picture is presented if the varieties are compared from the standpoint of loss of larvae rather than from that of number of cysts; whereas, for example, the number of cysts formed on Golden Wonder is 140% greater than the number formed on Epicure, only 7% more larvae survive in the former than the latter case. If the enormous loss of larvae indicated by this and other work occurred by death within the roots, it is certain that it would have been noticed by some of the many workers who have examined roots for eelworms; it is therefore reasonable to suppose that most of the larvae actually fail to reach the roots, a conclusion which would be in agreement with the repeated observation that the number of cysts varies with the heaviness of the medium (Reinmuth, 1929, Triffitt, 1930, Boyd, 1943). Not only would a less extensive root system be more difficult for the larvae to find, but, presumably, the production of root excretions which probably enable the roots to be found would also be less. If failure to reach the roots is indeed the cause of the enormous loss of larvae, the small differences in survival rates which Gemmell's data indicate for the forms he examined may well be due to small differences in this same factor, namely, the ability of the larvae to find the roots; it would certainly seem more reasonable to assume that this is the case until much more convincing evidence is produced to the contrary.

The smaller infestation secured in some cases might easily explain another of Gemmell's findings, viz., the fact that in Epicure and Doon Star cysts are found in which there is no trace of larvae, eggs, or egg cases. If adult males are reduced in numbers to the same extent as adult females (cysts), there may not be a sufficient number available to fertilize the females. Clearly, for a plant pot of a given size, there must be a minimum number of males available in order that all the females shall be fertilized and this number will be a function of the number of females and the size of the territory in which they are dispersed. Gemmell's observation about the cyst contents may therefore be a natural corollary of the fact that, in some cases, the numbers of mature animals is very much reduced. The cysts examined in the present work were obtained from plants grown in the field and probably subjected to a much higher infestation than in Gemmell's experiments; it is therefore not without significance that although the number of larva-producing Doon Star cysts was considerably less than in the other cases, there was not a single cyst which did not contain eggs.

The difference in the production of larvae by cysts of the same volume is attributed by Gemmell to the 'resistance' of the host plant. 'Examination of cysts from the "resistant" varieties has not indicated any notable reduction in egg content when compared with the susceptible varieties but, in the former case, a large number of the contained eggs are incapable of hatching.' I have already pointed out that, in the present case, Doon Star cysts were found to contain far fewer eggs than cysts of the other sorts. Possibly the different cultural conditions accounts for this difference in our findings; on the other hand, as no figures are presented to support this important statement it is possible that Gemmell's remarks may have been based on general observation of cyst contents rather than on egg counts. Nevertheless, although the number of eggs was less in the case of Doon Star, the number hatching was, relatively, even less; there is therefore a difference in hatching rate. I have suggested above that this difference may be due to factors concerned with the response of cysts *as units*. Gemmell suggests that, owing to the 'resistance' of the host, there is a decrease in the development of larvae, and speaks of eggs which 'contain only disorganized contents', unfortunately, again without presenting any details as to their frequency. My own examination failed to show any 'complete disorganization' among Doon Star eggs. Possibly there may be some

more subtle difference; a technique for distinguishing living and dead eggs would be a great help in this connexion. But even if it were shown that there are differences in the frequency of 'disorganized' eggs, it would not necessarily mean that these were due to degrees of 'resistance' on the part of the hosts. The factors influencing cyst size, egg number, etc., are almost unknown; their importance can be appreciated from the demonstration, in the present work, that the number of eggs per unit of cyst volume, may increase with cyst size, in a *single cyst type*.

Differences in cyst size are also attributed by Gemmell to the resistance of the host plants; although Epicure and Doon Star reach maturity sooner than Golden Wonder, there is little if any difference between Doon Star and Majestic, so that it is hardly likely, he argues, that the size of the cysts is due to the lateness or otherwise of the variety. In any case, 'between the last addition of larvae and the death of the earliest variety Epicure there was ample time for the full life cycle of the nematode'. The maximum size of the largest Epicure cysts is the same as the maximum on Golden Wonder, but the distribution of sizes within the range varied greatly. 'Thus, if the size of the cyst were solely a function of the length of time spent by the nematode on the host, the maximum size of the cysts would differ with the different dates of the ripening of the hosts. Since this is not so, it appears that cysts borne on Epicure have sufficient time to reach their full development, and the fact that the average size is smaller than on Golden Wonder indicates the existence of this effect of plant resistance.'

In the present study, although the largest Doon Star cysts were not quite as large as the largest cysts of the other sorts, they approach them closely; but the average size is much less. It is hardly to be expected however that the size of the cyst would be determined 'solely' by the length of time spent on the plant, and that an evergreen potato plant would produce evergrowing eelworm cysts! It is much more probable that an eelworm cyst will mature normally after it has reached a certain stage, and that under certain conditions it may reach this stage sooner or be stimulated to mature sooner; for example, Godfrey & Oliveira (1932) have shown that, under identical conditions, the root-knot eelworm matures in 19 days in the cowpea and 35 days in the pineapple. The fact that large Doon Star cysts are almost as large as the largest of the other sorts appears to me to be either evidence of variation of a genetical nature in the eelworm population, or an argument against 'resistance' rather than in favour of it. Moreover, while it may be true that Doon Star and Majestic plants mature at about the same time, in the sense that they begin to die down at the same time, they may not show the same seasonal variation in other respects. It is well known that potato varieties differ very considerably among themselves in, for example, their photoperiodism (for references, see Driver & Hawkes, 1943); this affects not only the time at which they die down but the whole of their metabolism. It is much more likely that cyst size is determined by factors of this sort than by 'resistance' in the sense employed by Gemmell;* and these factors should certainly be explored before his hypothesis is accepted.

I am very grateful to Prof. A. D. Hobson for the encouragement he gave me during the course of this work.

* Experiments still in progress already suggest that this is the case.

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A note on the relation of nematodes (*Tylenchus tritici*) to the development of the bacterial disease of wheat caused by *Bacterium tritici*

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(With 4 Text-figures)

Inoculation experiments using bacteria alone did not produce the bacterial disease of wheat: inoculations with mixed bacteria and nematodes were successful. The bacteria within the nematode galls retain their viability for 2½ years. The bacterial disease is more damaging to the wheat plant than the nematode infection.

Introduction

The bacterial disease of wheat caused by *Bacterium tritici* was reported from India (Hutchinson, 1917; Chaudhuri, 1935), Egypt (Fahmy & Mikhail, 1925) and Western Australia (Carne, 1926). Fahmy & Mikhail (1925) found that inoculations by bacteria alone were not successful, and Carne (1926) found that bacteria were carried into plants by nematodes. However, Chaudhuri (1935) at Lahore, India, concluded from inoculation experiments in field plots that bacteria alone could cause the disease and that the presence of nematodes was not necessary. He states that 'Nematode infection always takes place in the soil, usually at the time of germination of the seeds, but bacterial infection may be brought about at any stage of the growth of the wheat plant', and suggests that 'the infection in the latter case spreads from one plant to another by rubbing as when the wind blows'.

The writer found this disease in 1936 at Tinghsien, Hopei, and again received specimens in 1940 collected and kindly sent to him by Messrs C. S. Tsuang and K. L. Yen of the Agricultural Station of the National Bureau of Agricultural Research at Kweichow. The diseased plant is characterized by a curling of the emerging leaves with gummy exudations. The culms may grow until heading, and the spike is usually narrower and shorter than the normal one. All or many of the spikelets, instead of producing normal grains, may be full of a bacterial mass, and the glumes are externally covered with a bright yellowish slimy bacterial growth. Not infrequently the diseased spikes before heading become curled and distorted. The symptoms of the disease and the cultural behaviour of the causal organism agree closely with those reported in other countries.

Experimental

This bacterial disease has been found so far in China only in Hopei and Kweichow where the nematode disease is also prevalent. In 1936 field and pot experiments were conducted at Peiping to determine the role of the nematode in the development of the bacterial disease. In the field experiments susceptible wheat seeds were sown in four plots, each with fifty 3 ft. rows (1) inoculated with material from galls containing bacteria associated with nematodes, (2)

inoculated with the powdered bacterial mass from the diseased specimens, (3) inoculated with nematode galls and the powdered bacterial mass, (4) control, not inoculated.

Similarly susceptible wheat seeds were sown in pots in four series, each consisting of ten pots. The results obtained in May 1937 were as follows: all the controls remained healthy; no disease was produced when bacteria were inoculated alone; only a few heads covered with yellow exudations as well as many heads with nematode galls were produced when nematode galls were inoculated alone; a large number of diseased heads, but few with nematode galls, were produced when bacteria and nematode galls were inoculated. Unfortunately, data of these experiments were lost in the war. This paper presents results of experiments conducted at Kunming since 1940 to determine the role of the nematode in the development of the disease, the viability of the bacteria associated with the nematode galls and the distribution of infection of the wheat plant when inoculated with galls containing bacteria associated with nematodes.

In October 1940 inoculation experiments were conducted in pots in four series: (1) control—not inoculated, (2) inoculated with pure culture of bacteria alone, (3) inoculated with galls containing bacteria associated with nematodes; and (4) inoculated with bacteria plus nematode galls and pure culture of the bacteria. The results of the experiments are given in Table 1. It is evident from this table that the bacterial disease was produced only when the galls were used as inocula.

TABLE 1. *Results of inoculation experiments at Kunming, 1940–1*

Inocula	Total spikes	Spikes attacked by bacteria	Spikes attacked by nematode
Nematode galls	227	23	6
Nematode galls and pure culture of bacteria	181	6	82
Pure culture of bacteria	—*	0	0
Not inoculated	109	0	0

* Since all the plants were free from disease the total number was not counted.

TABLE 2. *Results of inoculation experiments at Kunming, 1941–42*

	Wheat variety	Soil treatment	Inocula	Total spikes	Spikes attacked by nematode		Spikes attacked by bacteria	
						%		%
Field experiments	T.H. 3758	Not sterilized	Nematode galls:					
			Kweichow (1939)	229	26	11·3	0	0
			Kweichow (1940)	263	40	15·2	1	0·38
			Kweichow (1941)	527	115	21·8	0	0
Pot experiments	T.H. 3758	Not sterilized	Kweichow (1939)	49	14	28·5	0	0
			Kunming (1941)	34	25	73·0	0	Only on young leaves
		Sterilized	Kweichow (1939)	24	5	20·8	3	12·5
			Kunming (1941)	28	26	92·8	1	3·5
			Pure culture of bacteria	105	0	—	0	—
			Powdered bacterial mass	116	0	—	0	—

THE ROLE OF THE NEMATODE IN THE DEVELOPMENT OF THE DISEASE

For 3 years inoculation experiments with bacteria alone, either from pure culture or from scrapings of diseased specimens, using Chaudhuri's spraying method, were not successful. Hypodermic injections of the bacterial suspension into young seedlings in pots also failed to produce the disease even under bell jars with a considerable amount of moisture. The bacterial disease was produced only when the galls containing bacteria associated with nematodes were used as inocula with or without addition of the bacteria.

In November 1941 inoculation experiments were repeated in the same manner with the exception that the soil of some of the pots was sterilized at 30 lb. for half an hour twice in two successive days. The inocula used were the galls collected in different years, pure cultures of the bacteria and the powdered bacterial mass from scrapings from diseased specimens.

It is shown again in Table 2 that the bacterial disease was produced only by the inoculation with the galls. The results show that soil sterilization had no definite effect on the development of nematode disease, while the treatment did increase slightly the

TABLE 3. *Results of inoculation experiments at Kunming, 1942–3*

Wheat variety	Nematode gall treatment	Nematode galls	Total plants	Total spikes	Plants attacked by nematodes		Spikes attacked by nematodes		Plants attacked by bacteria		Culms and spikes attacked by bacteria	
						%		%		%		%
Ding-fang no. 1	No treatment	Kweichow (1939)	39	186	5	12·8	6	3·2	0	0	0	0
		Kweichow (1940)	40	155	7	17·5	13	8·3	1	2·5	1	0·6
		Kweichow (1941)	37	135	26	70·2	50	37·0	23	62·1	32	23·6
		Ding-fang (1941)	43	165	31	72·0	58	35·1	24	55·8	45	27·2
		Kunming (1942)	37	155	21	56·7	31	20·0	23	62·1	51	32·9
C.B.A.R. no. 412	No treatment	Kunming (1942)	19	102	14	73·6	20	19·6	10	52·6	20	19·6
	Surface disinfected	Kunming (1941)	19	102	1	5·2	1	0·9	14	73·6	31	30·3
		Kunming (1942)	23	94	14	60·8	25	26·5	12	52·1	19	20·2

TABLE 4. *Distribution of infection of wheat plants when inoculated with galls containing bacteria associated with nematodes*

	No. of plants	%	No. of culms	No. of heads or culms infected	%	
Plants with galls alone	103	32·2	493	176	35·7	
Plants attacked by bacteria alone	102	31·9	510	231	45·3	57% of the infected culms fail to head
Plants simultaneously attacked by nematode and bacteria	115	35·9	646	458	70·8	
Totals	320		1649	865	52·5	

Figs. 1–4. Sections of infected growing points, showing the distribution of nematodes and bacteria.

percentage of infection in the case of the bacterial disease.

Further investigations were made to determine whether the galls carry with them the causal bacteria externally or internally. Nematode galls from Kweichow were surface-disinfected in 95% alcohol for 1 min. and in mercuric chloride 1 : 1000 for 1 min., dried and sown simultaneously with wheat seeds in sterilized soil in pots in equal numbers.

The results indicate that the disinfectant had no effect on the percentage of infection, hence the bacteria seem to be carried mainly within the nematode galls.

Viability of bacteria associated with the nematode galls

It is shown in Tables 2 and 3 that the bacterial disease appeared in the spring of 1942 and 1943 when the galls collected in 1940 were used as inocula in the autumn of 1941 and 1942 respectively. This means that the bacteria associated with the nematode galls are still alive after being dormant $2\frac{1}{2}$ yr.

Histological studies

Examination of the growing points of nematode-infected wheat plants about 10 cm. high, shows that tissues invaded by the nematodes may contain no bacteria (Figs. 2, 3), but those with bacteria all contain the nematodes at the same time (Figs. 1–4). The same occurs in the developing spikes. In the leaf sections, however, only bacteria are observed.

Distribution of infection of wheat plants when inoculated

As a single plant or a single spike may provide both nematode galls and bacterial infection at the same time, a study was made of the distribution of infection of the wheat plant by both diseases. An analysis of the data of 320 wheat plants in pot experiments (Table 4) shows that the bacterial disease is more damaging than the nematode disease, for over 50% of the bacteria-infected culms are prevented from heading.

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A mosaic disease of cowpea (*Vigna sinensis* Endl.)

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(With Plate 13)

A mosaic disease of cowpea (*Vigna sinensis* Endl.), prevalent in the bean-growing regions of China, is described.

The characteristic symptoms of the disease are conspicuous mosaic patterns, deformation and distortion of leaves and stunting of the plant.

The cowpea mosaic virus is transmitted by *Aphis rumicis* L., *Macrosiphum pisi* Kalt. and *Aphis gossypii* Glov. It is also transmitted by artificial juice inoculation. The virus is seed-borne. It withstands ageing *in vitro* for 3 days at 22° C. Its inactivation temperature is approximately 62° C.; its tolerance to dilution is about 1 : 3000.

Cowpea mosaic virus infects lima bean and Adzuki bean in addition to cowpea.

The relation of this virus to cowpea mosaic virus described by McLean and asparagus-bean mosaic virus described by Snyder is discussed.

Introduction

In 1939 a mosaic disease of cowpea (*Vigna sinensis* Endl.) was reported in China (Yu, 1939). It was very prevalent along the Yangtze valley and might produce heavy losses in susceptible varieties.

Preliminary cross-inoculation experiments made in 1933 indicated that the cowpea mosaic virus is not related to either common bean mosaic virus or soybean mosaic virus. Investigations of the cowpea mosaic virus were therefore made from 1933 to 1937 in Nanking, China, and the results of studies on symptomatology, transmission, host range and properties of the virus are now given in this paper.

Review of literature

Elliot (1928) first reported a cowpea mosaic in Arkansas in 1921. Smith (1924) was the first to transmit the cowpea mosaic virus both by mechanical inoculation and by insect. He claimed almost 100% transmission with the leaf beetle (*Ceratoma trifurcata*) fed for one day on diseased cowpea plants and then transferred to healthy plants. Gardner (1927) reported seed transmission of cowpea mosaic in the varieties Progress White and Arlington.

In 1941 McLean published the results of his studies on mosaic of cowpea (*Vigna sinensis* Endl.) and Snyder (1942) reported a seed-borne mosaic disease of asparagus bean (*Vigna sesquipedalis* Wight). Vasudeva (1942) also reported a mosaic disease of cowpea in India.

Methods and materials

The cowpea mosaic virus used in this work came from a mature infected cowpea plant found in the university garden, Nanking. After the virus had been established on cowpea plants in the greenhouse by mechanical inoculation, successive transfers were made to healthy seedlings at frequent intervals to provide a constant source of fresh inoculum.

Mechanical inoculations were made by dusting the leaves with powdered carborundum and rubbing lightly with a piece of cheese cloth soaked with the inoculum. A few minutes after the leaves were rubbed, most of the inoculum was removed by spraying the leaves with water.

The cowpea used for inoculation was a very susceptible variety, the Long Pod, which is commonly grown in China. Seedlings with four to six leaves served as test plants. In greenhouse tests to determine the host range of the virus, mechanical methods of transmission and insects were both used.

The temperature at which these tests were conducted ranged from 25 to 32° C. It was found that the symptoms of the disease might be greatly suppressed when the temperature was below 22° C., especially at low light intensities.

Symptoms of the disease

The symptoms of cowpea mosaic differ in different varieties, and, in the same variety, vary with the type of leaf, time of infection, climatic conditions and individual difference in plants.

The first symptom is a clearing of the veins, which starts at or near the base of the leaf and gradually spreads over the entire leaf. In certain cowpea varieties, this vein-clearing is very pronounced (Pl. 13, fig. 1A, B). After 10–12 days the early symptoms on the young inner leaves change gradually into a very conspicuous mottling. The irregular light and dark green areas between the veins may cause little or no distorting of the leaf surface of the less susceptible cowpea varieties under field conditions (Pl. 13, fig. 2A).

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Chlorotic areas are usually well demarcated from the still green tissue. Sometimes almost the whole leaflets are chlorotic; at others there are only small chlorotic blotches (Pl. 13, fig. 3B). The irregular patches of light green often contain islets of normal green, which are often parallel to the veins (Pl. 13, figs. 2B and 3A, B). Accompanying the mosaic symptoms there is frequently a slight convex cupping, arching or inward rolling of the margin of the leaflets (Pl. 13, figs. 1A, B and 3B). The affected leaves are often puckered and show deep marginal indentations (Pl. 13, fig. 4A, B.). In some varieties and in certain climatic conditions, yellowish primary lesions are formed, which turn dark brown towards the end of the growing season. Dark reddish brown discoloration may also be produced on the veins.

The symptoms are usually most severe in the upper portion of the plant. The top leaves, besides showing the symptoms described above, are stunted, greatly misshapen, and puckered, with dark green areas along the veins. The affected leaflets tend to be asymmetrical, reduced in size, wrinkled, crinkled, deformed, twisted and curled either inward or downward about the margin. These symptoms are illustrated by figs. 5–7 in Pl. 13.

In addition to these different leaf symptoms, there is a general stunting of the plants, shortening of the internodes, and a tendency towards excessive branching; deformation of the pods is common on susceptible varieties such as Long Pod.

Transmission

Seed. In 1934 seed was collected from a planting of Long Pod in which all the plants were affected with mosaic. These seeds were planted in April 1935. From 600 of these, 483 seedlings were produced, of which fifty-two developed symptoms of mosaic in the first compound leaf, i.e. 10·8% of the seeds were infected with mosaic virus. In 1935 seed was collected from plants which either originated from infected seed or had contracted the disease during the growing season. Of 892 plants produced by seed of the former and 644 by that of the latter, seventy-two and forty-nine respectively showed mosaic when they were at the third trifoliate leaf stage. These results confirm previous work that the cowpea mosaic is seed transmitted (Gardner, 1927; McLean, 1941; Snyder, 1942).

Mechanical inoculation. The cowpea mosaic virus can be transferred from diseased to healthy seedlings by mechanical inoculation without the help of abrasives, but the percentage of infection is greatly increased when the abrasive is used. The first symptom of the disease appeared on the inoculated leaflets in from 7 to 15 days. In most plants the inoculated leaflets became curled along the leaf margin and more or less distorted. The newly developed leaves produced the characteristic mosaic symptoms usually with little or no distortion.

Insect transmission. All the experiments were carried out in insect-proof cages kept in a gauze-protected greenhouse. The insects used were the black aphis (*Aphis rumicis* L.), pea aphis (*Macrosiphum pisi* Kalt.) and the cotton aphis (*Aphis gossypii* Glov.). Twenty or more non-infective aphides were allowed to feed for 24 hr. on a diseased cowpea in an insect-proof cage. Healthy test seedlings were then introduced into the cage and the infected plant was cut down; the aphides then moved across to the test plants with a minimum of handling. All the plants were sprayed with nicotine sulphate solution 24 hr. after the aphides had moved. Healthy cowpea seedlings colonized with non-infective aphides served as controls. The results are summarized in Table 1 and show that all three species of aphis can transmit the virus. The symptoms were identical with those produced by mechanical inoculation; they appeared in from 4 to 7 days after transfer of the aphides, and soon after this the terminal leaf buds and trifoliate leaves were malformed. As these three species of aphis are common in fields of cowpea, they are assumed to be the vectors in nature, and no further search was made for other vectors.

Table 1. *Results of cage experiments on insect transmission of cowpea mosaic*

	Percentage of infection		
Insects used	1935	1936	1937
Black aphis (*Aphis rumicis*)	60	70	70
Pea aphis (*Macrosiphum pisi*)	100	100	100
Cotton aphis (*Aphis gossypii*)	100	80	100

Properties

Longevity in vitro. The leaves of infected cowpea were ground to a fine pulp, and the juice extracted by squeezing through cheese-cloth. The undiluted juice was then stored in a stoppered Erlenmeyer flask in the dark in an incubator held at 22° C. At the end of each 24 hr., for a period of 5 days, a small quantity of the juice was removed, diluted with about 2 parts of water, and used to inoculate ten small cowpea seedlings.

Inactivation temperature. 2 c.c. samples of undiluted juice, from recently infected cowpeas, were put in small thin-walled test-tubes, and heated in a constant-temperature water-bath for 10 min. The extract was then rapidly cooled and diluted with about 2 vol. of water before inoculation.

Effect of dilution. Dilutions of sap were made with distilled water and were tested by mechanical inoculation with the aid of carborundum.

The results are shown in Table 2: with a longevity *in vitro* of about 3 days, a thermal inactivation point

of 62° C., and a dilution end-point of 1 in 3000, they agree closely with the properties given by Snyder (1942) for his asparagus-bean mosaic virus.

Host range

By means of both mechanical inoculation and aphid transmission, the cowpea mosaic virus was transmitted to lima bean (*Phaseolus lunatus macrocarpus*) and adzuki bean (*Phaseolus angularis* var. Chu-Sha). On lima bean, the virus produced typical mosaic symptoms, occasionally accompanied by yellow blotches. On adzuki bean, patches of light green were often formed as elongated blotches parallel with the veins of the leaflets, which frequently showed wrinkling and marginal indentation.

Identity of the virus

Several viruses are known to produce necrotic lesions on cowpeas. These viruses are lima-bean mosaic virus (McClintock, 1917; Harter, 1938), tobacco ring-spot virus (Wingard, 1928), tobacco-necrosis virus (Smith & Bald, 1935), celery mosaic virus (Price, 1935*a*, *b*), cucumber mosaic virus (Price, 1935*a*, *b*), alfalfa mosaic virus 1 (Zaumeyer, 1938; Black & Price, 1940), alfalfa mosaic virus 1 A and 1 B, alfalfa mosaic virus 2 (Zaumeyer, 1938), and potato calico virus (Black & Price, 1940).

Table 2. *Longevity* in vitro, *inactivation temperature and tolerance to dilution of cowpea mosaic virus*

Longevity in vitro

Age (hr.)	Plants inoculated	Plants infected	Age (hr.)	Plants inoculated	Plants infected
0	20	16	72	20	3
24	20	13	96	20	0
48	20	9	120	20	0

Inactivation temperature, 10 min.

Temp. ° C.	Plants inoculated	Plants infected	Temp. ° C.	Plants inoculated	Plants infected
50	20	15	58	20	11
52	20	17	60	20	5
54	20	14	62	20	3
56	20	10	64	20	0

Tolerance to dilution

Dilution	Plants inoculated	Plants infected	Dilution	Plants inoculated	Plants infected
0	20	13	1 : 1500	20	4
1 : 10	20	16	1 : 2000	20	7
1 : 100	20	14	1 : 2500	20	0
1 : 500	20	8	1 : 3000	20	1
1 : 1000	20	11	1 : 3500	20	0

No infection was obtained by mechanical inoculations in the following plants: *Cucumis sativus* L., *Solanum Melongena* L., *S. tuberosum* L., *Capsicum annuum* L., *Lycopersicum esculentum* Mill., *L. pimpinellifolium* Dunal, *Petunia hybrida* Hort., *Datura stramonium* L., *Nicotiana tabacum* L., *Zinnia elegans* Jasq., *Apium graveolus* L., *Beta vulgaris* L., *Lens esculenta* Moench., *Pisum sativum* L., *P. sativum* var. *arvense* Poit., *Vicia faba* L., *V. sativa* L., *V. villosa* Roth., *Lupinus albus* L., *Lathyrus odoratus* L., *Arachis hypogaea* L., *Phaseolus vulgaris* L., *Dolichos Lablab* L., *Canavalia ensiformis* DC., *Lespedeza bicolor* Trucz., *Glycine Max* Merr., *Medicago sativa* L., *Melilotus alba* Desr., *Trifolium repens* L., *T. pratense* L. and *Pachyrhizus tuberosum* Spreng. These plants were tested only in a single experiment and the results cannot be regarded as conclusive. Unfortunately, no seed of asparagus bean was available to include in these host-range determinations.

Price (1934) obtained two strains of cucumber mosaic virus derived experimentally from a mild mottling strain of cucumber mosaic virus during serial passages in cowpeas. The *Y* strain differs from the original type virus and from all other strains in its ability to produce a systemic disease in Blackeye cowpea.

Severin & Henderson (1928) reported the natural infection of cowpea with the curly-top virus.

The viruses that have been reported unable to cause infection on cowpeas are: pea virus 1 (Stubbs, 1936), pea virus 2 (Stubbs, 1936; Zaumeyer & Wade, 1935), pea virus 2 B and 2 C (Stubbs, 1936), tobacco ring-spot virus (Stubbs, 1936), tobacco virus 1 (McLean, 1941), common-bean mosaic virus (Reddick & Stewart, 1918), white-clover mosaic virus,

white sweet-clove mosaic virus, alfalfa mosaic virus, red-clover mosaic virus (Zaumeyer & Wade, 1935), alfalfa mosaic virus 2 (Pierce, 1934), bean virus 1 (McLean, 1941; Pierce, 1934), and celery mosaic virus (Wellman, 1934). The writer has failed to infect cowpea with bean mosaic virus 1 and soybean mosaic virus.

Detailed studies of cowpea and asparagus-bean mosaic viruses have been made respectively by McLean (1941) and Snyder (1942), both in the United States of America. McLean's cowpea mosaic virus produces, on the highly susceptible cowpea varieties, a chlorosis generally associated with dwarfed slender growth and excessive branching. The virus is transmitted by mechanical inoculation and by four species of aphides, viz. *Macrosiphum solanifolii*, *M. pisi*, *Aphis gossypii* and an undetermined black aphis closely resembling *A. rumicis*. The virus is seed-borne. It has been transmitted to lima bean by manual inoculation and by the pea aphis. Transmissions of the virus to *Vicia faba*, *Pisum sativum*, *P. sativum* var. *arvense*, *Lathyrus odoratus*, *Phaseolus vulgaris*, *P. aureus*, *P. lunatus* var. Fordhook, *Lycopersicum esculentum*, *Solanum tuberosum*, *Petunia hybrida*, *Datura stramonium*, *Capsicum annuum*, *Zinnia elegans*, *Cucumis sativus*, *Nicotiana tabacum* and *N. glutinosa* were unsuccessful. The virus is inactivated when heated for 10 min. at 72–75° C., on ageing *in vitro* from 2 to 3 days and at a dilution of about 1 : 1000.

The symptoms produced by the asparagus-bean mosaic virus reported by Snyder (1942) on this bean consist of mosaic, downward rolling, mild rugosity or distortion of leaflets, vein-banding and stunting. The disease is transmitted both by mechanical inoculation and by pea aphis. The virus is seed-borne. The inactivation temperature of the virus is between 55 and 60° C., its longevity *in vitro* about 2 days, and its dilution end-point nearer 1 : 1000 than 1 : 3000. Infection of cowpea produces symptoms resembling those of cowpea mosaic. On common bean, the symptoms produced do not agree with those of common bean mosaic. It has been found that the asparagus-bean mosaic virus does not infect *Vigna catjung* Walp., *Soja Max*, *Pisum sativum*, *Medicago sativa* (common and Peruvian alfalfa), *Acer arietinum*, *Lathyrus odoratus*, *Melilotus alba*, *M. indica*, *Trifolium repens*, *T. pratense*, *Nicotiana tabacum* and *N. glutinosa*.

McLean (1941) pointed out that the symptoms produced by his cowpea mosaic virus are apparently similar to those caused by the two strains of cucumber mosaic virus obtained by Price (1934). Price's cowpea strain of cucumber mosaic virus, the cowpea-mottling strain, is classified by Holmes (1939) as a variety of cucumber mosaic virus and has not been found occurring naturally on cowpeas.

In a discussion of the relation of asparagus-bean mosaic virus to McLean's cowpea mosaic virus, Snyder (1942) pointed out that the former may differ from the latter by its failure to infect catjung, by differences between the thermal inactivation points, and by certain minor differences in the symptoms produced. He admitted that these differences are not large, and suggested that the two viruses are closely related or perhaps even strains of one virus.

Regarding the cowpea mosaic virus described in this paper, it is interesting to note that it closely resembles the asparagus-bean mosaic virus both in the symptoms produced and the properties exhibited. On the other hand, its host range closely resembles that of McLean's cowpea mosaic virus. The symptoms produced are also similar to those of McLean's virus in general. Since the symptoms and properties of the virus *in vitro* are liable to variations because of various factors, such as climatic conditions, differences in reactions of host varieties, time of infection, age of infected plants, etc., it is therefore reasonable to consider that all of these three viruses are closely related, if not identical.

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EXPLANATION OF PLATE 13

Fig. 1. Cowpea leaves showing pronounced vein-clearing.

Fig. 2. Cowpea leaves showing mosaic patterns of various forms.

Fig. 3. Cowpea var. Long Pod, showing characteristic mottling (A) and large mosaic patterns (B).

Fig. 4. Cowpea leaves showing puckering.

Fig. 5. Young trifoliate leaves of cowpea plants showing distortion and rugosity.

Fig. 6. Young trifoliate leaves of cowpea showing rugosity and marginal rolling.

Fig. 7. Young trifoliate leaves of cowpea var. Long Pod showing severe distortion and deformation.

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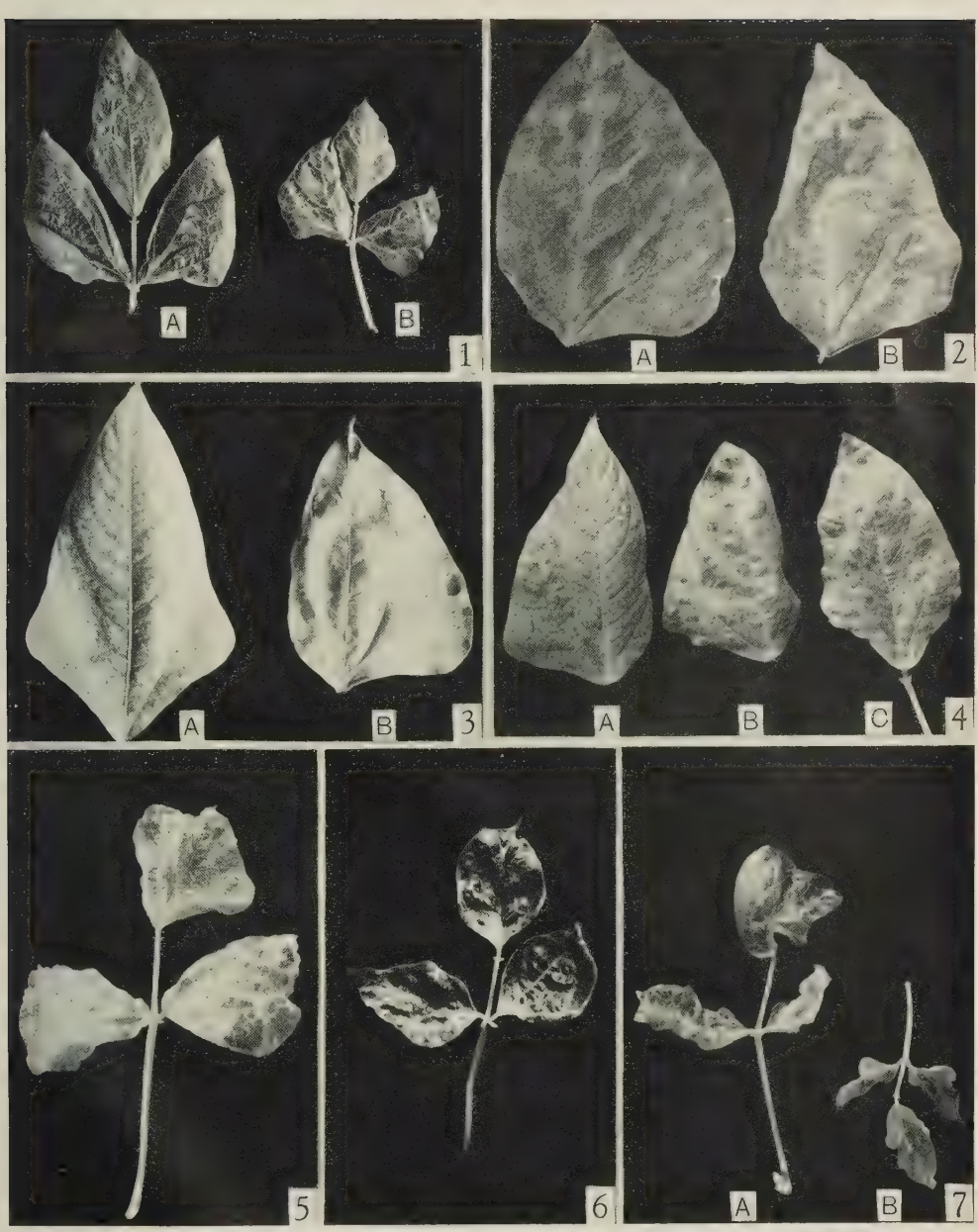

YU—A MOSAIC DISEASE OF COWPEA

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1933 Gadd, C. H., D.Sc., Tea Research Institute of Ceylon, St Coombs, Talawakelle, Ceylon.
1930 Galloway, L. D., M.A., 4 Hanway Place, London, W. 1.
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1944 Gemmell, A. R., B.Sc., M.S., Ph.D., Department of Botany, The University, Manchester 13.
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1920 Gimingham, C. T., O.B.E., B.Sc., F.R.I.C., Plant Pathology Laboratory, Milton Road, Harpenden, Herts. (President 1938–40; Vice-President 1937; Zoological Editor 1934–44; Council 1929–32.)
1941 Glasscock, H. H., M.Sc., Wye College, nr. Ashford, Kent.
1920 Glynne, Miss M. D., D.Sc., Rothamsted Experimental Station, Harpenden, Herts. (Council 1946– .)
1941 Goddard, W. H., D.I.C., Pest Control, Ltd., Harston, Cambridge.
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1921 Gray, Prof. P. H. H., M.A., D.Sc., Macdonald College, Quebec, Canada.
1929 Green, D. E., M.Sc., R.H.S. Laboratories, Wisley, Ripley, Woking, Surrey. (Council 1941–3.)
1939 Greenhill, A. W., Ph.D., Boron Agricultural Bureau, Clock Offices, 2 Station Road, Harpenden, Herts.
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1920 Gwynne-Vaughan, Prof. Dame Helen, G.B.E., D.Sc., LL.D., F.L.S., Birkbeck College, Chancery Lane, London, E.C. 4.
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1930 Hall, W. J., D.Sc., A.R.C.S., Imperial Institute of Entomology, 41 Queen's Gate, London, S.W. 7.
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1942 Harrison, J., M.A., Ph.D., Pharmaceutical Specialities (May and Baker), Ltd., Dagenham, Essex.
1938 Harvey, G. L., B.Sc., A.R.C.S., 12 Whitemore Road, Beckenham, Kent.
1927 Hatton, R. G., C.B.E., V.M.H., M.A., D.Sc., F.R.S., Director, East Malling Research Station, nr. Maidstone, Kent. (Council 1928.)
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1943 Heath, O. V. S., Ph.D., D.I.C., A.R.C.S., Imperial College Laboratory, Rothamsted Experimental Station, Harpenden, Herts.
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1938 Henderson, F. Y., D.Sc., Director, Forest Products Research Laboratory, Princes Risborough, Bucks.
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1942 Hewlett, P. S., B.Sc., Pest Infestation Laboratory, Biological Field Station, Slough, Bucks.
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1946 Hickley, H. M. H., B.Sc., Ministry of Food, Infestation Division, 58 High Holborn, London, W.C. 1.
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1920 Hiscox, Miss E. R., M.Sc., National Institute for Research in Dairying, Shinfield, Reading.
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1928 Hughes, A. W. McKenny, D.I.C., British Museum (Natural History), South Kensington, London, S.W. 7.

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1938 Hunter-Smith, J., M.B.E., B.Sc., N.D.A., Principal, Herts. Institute of Agriculture, Oakland, St Albans, Herts.
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1943 Isaac, I., B.Sc., Ph.D., The Grammar School, Cheltenham, Glos.
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1927 Jacobs, S. E., Ph.D., Bacteriology Department, Imperial College of Science, South Kensington, London, S.W. 7. (Council 1942–3.)
1945 Jacot-Guillarmod, Mrs C., M.A., B.Sc., P.O. Mamathes, via Teyateyaneng, Basutoland, South Africa.
1927 Jary, S. G., B.A., Wye College, nr. Ashford, Kent. (Council 1934–6.)
1945 Jefferies, P., Mitchell Cotts and Co., Ltd., Winchester House, Old Broad Street, London, E.C. 2.
1942 Jenkin, Prof. T. J., D.Sc., Welsh Plant Breeding Station, Agricultural Research Building, Penglais, Aberystwyth.
1942 Johnson, G. C., St Regis, Montague Road, Cambridge.
1938 Johnson, L. R., M.Sc., Department of Agriculture, The University, Leeds 2.
1945 Johnson, Miss M. D. A., B.Sc., School of Agriculture, Cambridge.
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1940 Jones, F. G. W., B.A., School of Agriculture, Cambridge.
1927 Joseph, E. G., B.Sc., Redcot, Swanley, Kent.
1935 Kearns, H. G. H., B.Sc., Ph.D., Research Station, Long Ashton, nr. Bristol. (Council 1939–41.)
1940 Keay, Miss M. A., M.A., Ph.D., Imperial Bureau of Plant Breeding and Genetics, School of Agriculture, Cambridge.
1946 Kennedy, J. S., M.Sc., Ph.D., Entomological Field Station, Storey's Way, Cambridge.
1932 Kerr, H. W., M.Sc., Ph.D., Director, Bureau of Sugar Experiment Stations, Brisbane, Australia.
1940 Keyworth, W. G., B.Sc., Ph.D., D.I.C., A.R.C.S., East Malling Research Station, nr. Maidstone, Kent.
1907 King, L. A. L., M.A., Ivy House, Ockbrook, Derby.
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1946 Large, E. C., B.Sc., A.R.I.C., Seale-Hayne Agricultural College, Newton Abbot, Devon.
1933 Leach, R., B.A., Byrns Lodge, Grantchester, Cambridge.
1934 Lean, O. B., B.Sc., D.I.C., Imperial Chemical Industries, Ltd., Jealott's Hill Research Station, Bracknell, Berks. (Council 1940–2.)
1945 Lee, O. M., School of Agriculture, Serdang, Selangor, Malaya.
1926 Le Pelley, R. H., B.Sc., Ph.D., D.I.C., A.R.C.S., Department of Agriculture, Nairobi, Kenya, East Africa.
1943 Libbey, R. P., B.Sc., Prospect Cottage, Eldwick, Bingley, Yorks.
1946 Lloyd, A. J., B.Sc., Ph.D., 128 Whiteladies Road, Bristol 8.
1920 Lloyd, L., D.Sc., Zoological Department, The University, Leeds 2.
1946 Lush, G. B., B.Sc., Boots Pure Drug Co., Ltd., Lenton Experimental Station, Lenton House, Nottingham.
1931 McClean, A. P. D., D.Sc., Division of Botany and Plant Pathology, Department of Agriculture, 590 Vermeulen Street, Pretoria, South Africa.
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Orig. Macdougall*, Prof. R. S., M.A., D.Sc., Ivy Lodge, Gullane, E. Lothian, Scotland. (Vice-President 1914–19; Council 1908–13.)
1929 Macgill, Miss E. I., D.Sc., Linkfield, Hawthorn Road, Denton, Manchester.
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1925 Macleod, D. J., M.A., Dominion Laboratory of Plant Pathology, Fredericton, New Brunswick, Canada.
1938 Macleod, J., D.Sc., Cooper Field Research Station, Little Gaddesden, Berkhamsted, Herts.

1945 Maggs, D. H., B.Sc., Oil Palm Research Station, Agricultural Department, Benin City, Nigeria.
1920 Mangham, Prof. S., M.A., University College, Southampton.
1917 Mann, H. H., D.Sc., F.R.I.C., F.L.S., Woburn Experimental Station, Husborne Crawley, Bletchley.
1938 Marsh, R. W., M.A., Research Station, Long Ashton, nr. Bristol. (Assistant Editor 1945; Editor 1946– .)
1930 Martin, H., D.Sc., A.R.C.S., F.R.I.C., Research Station, Long Ashton, nr. Bristol. (President 1941–2; Vice-President 1938; Council 1936–8.)
1940 Martin, J. T., D.Sc., F.R.I.C., Plant Pathology Laboratory, Milton Road, Harpenden, Herts. (Council 1945– .)
1922 Mason, E. W., M.A., M.Sc., F.L.S., Imperial Mycological Institute, Kew, Surrey. (Vice-President 1943–4; Council 1943–5.)
1927 Massee, A. M., D.Sc., East Malling Research Station, nr. Maidstone, Kent. (Council 1932–4.)
1946 Matthews, R. E. F., M.Sc., Plant Virus Research Station, School of Agriculture, Cambridge.
1938 Meggitt, F. J., M.Sc., Ph.D., University College, Rangoon.
1933 Meiklejohn, Miss J., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.
1943 Mellor, H. C., B.Sc., A.R.C.S., Bayer Products, Ltd., Agricultural Laboratory, 272 Colney Hatch Lane, Friern Barnet, London, N. 11.
1941 Metcalfe, C. R., M.A., Ph.D., Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey.
1923 Miles, H. W., D.Sc., Ph.D., Woodthorne, Wergs Road, Tettenhall, Wolverhampton. (Vice-President 1939, 1943–4; Council 1937–9, 1943–5.)
1941 Milne, A., M.A., Ph.D., Department of Agriculture, King's College, Newcastle-on-Tyne 2.
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1940 Montgomery, H. B. S., B.A., Ph.D., D.I.C., East Malling Research Station, nr. Maidstone, Kent.
1932 Moore, Miss E. S., B.Sc., Ph.D., Tobacco Research Laboratory, Balfour, Cape Province, South Africa.
1946 Moore, Miss F. J. H., B.Sc., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.
1940 Moore, M. H., M.Sc., East Malling Research Station, nr. Maidstone, Kent. (Assistant Secretary 1946; Council 1945.)
1928 Moore, W. C., M.A., Plant Pathology Laboratory, Milton Road, Harpenden, Herts. (Vice-Presiden 1945; Treasurer 1940–6; Council 1938–9.)
1941 Morison, G. D., B.Sc., Ph.D., Advisory Entomological Department, Marischal College, Aberdeen.
1922 Morland, D. M. T., M.A., Seale-Hayne Agricultural College, Newton Abbot, Devon.
1920 Morris, H. M., M.Sc., Agricultural Department, Nicosia, Cyprus.
1920 Mosley, F. O., F.L.S., Research Laboratory, The Nurseries, Uxbridge, Middlesex.
1946 Muggeridge, J., B.Sc., Cawthron Institute, Nelson, New Zealand.
1945 Munro, J., B.Sc., National Institute of Agricultural Botany, Huntingdon Road, Cambridge.
1914 Munro, Prof. J. W., M.A., D.Sc., Zoology Department, Imperial College of Science, South Kensington, London, S.W. 7. (Vice-President 1933; General and Zoological Secretary 1928–32; Zoological Editor 1933; Council 1922–4, 1927.)
1938 Musgrave, A. J., B.Sc., D.I.C., British Leather Manufacturers' Research Association, 1–6 Nelson Square, London, S.E. 1.
1925 Muskett, Prof. A. E., D.Sc., A.R.C.S., Plant Disease Division, Queen's University, Elmwood Avenue, Belfast, Northern Ireland.
1937 Nattrass, R. M., B.Sc., Ph.D., D.I.C., Scott Agricultural Laboratories, Department of Agriculture, P.O. Box 338, Nairobi, Kenya, East Africa.
1928 Newton, H. C. F., A.R.C.S., D.I.C., Harper Adams Agricultural College, Newport, Salop. (Council 1938–40.)
1943 Newton, Miss L., M.Sc., Ph.D., Botanical Department, University College of Wales, Aberystwyth.
1944 Noble, Miss M., B.Sc., Ph.D., Seed Testing and Plant Registration Station, East Craigs, Corstorphine, Edinburgh 12.
1946 Notley, F. B., M.Sc., A.I.C.T.A., Coffee Research Station, Lyamungu, Moshi, Tanganyika Territory.
1944 Ogg, W. G., M.A., B.Sc., Ph.D. F.R.S.E., Director, Rothamsted Experimental Station, Harpenden, Herts.
1923 Ogilvie, L., M.A., M.Sc., Bracken Hill, Leigh Woods, Bristol 8.
1925 Oldham, J. N., B.Sc., Ph.D., Research Institute in Animal Pathology, Royal Veterinary College, The Beeches, Streatley, Berks. (Council 1934.)
1940 Ordish, F. G., Dip.Hort., Plant Protection, Ltd., Yalding, Kent.
1946 Owen, H., B.Sc., West African Cacao Research Institute, Tafo, Gold Coast, B.W.A.
1943 Oxley, T. A., B.Sc., A.R.C.S., Pest Infestation Laboratory, Biological Field Station, Slough, Bucks.

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1941 Parker-Rhodes, A. F., B.A., 132 Priory Lane, London, S.W. 15.
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1946 Peace, T. R., M.A., Forest Research Station, Alice Holt, Farnham, Surrey.
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1946 Peet, W. V., B.Sc., Ph.D., Coniston, Station Road, Epping.
1914 Petherbridge, F. R., M.A., School of Agriculture, Cambridge. (Vice-President 1940–1; Council 1939–41.)
Orig. Pethybridge,* G. H., M.A., B.Sc., Ph.D., Penlee, Harleigh Road, Bodmin, Cornwall. (Vice-President 1926–7; Council 1912–13, 1925–7.)
1946 Peverett, L., London Fumigation Co., Ltd., Marlow House, Lloyd's Avenue, London, E.C. 3.
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1946 Plaut, M., D.Phil., Agricultural Experiment Station, Rehoboth, Palestine.
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1942 Prentice, I. W., B.Sc., Ph.D., East Malling Research Station, nr. Maidstone, Kent.
1946 Preston, A. P., N.D.H., East Malling Research Station, nr. Maidstone, Kent.
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1946 Rainey, R. C., B.Sc., Ph.D., A.R.C.S., D.I.C., Empire Cotton Growing Corporation, Cotton Experiment Station, P.O. Box 95, Barberton, Eastern Transvaal, South Africa.
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1914 Roberts, A. W. Rymer, M.A., Applegarth, Cartmel, Grange-over-Sands, Lancs.
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1946 Satyanarayana, P., M.Sc., A.R.I.C., East Malling Research Station, nr. Maidstone, Kent.
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1941 Selman, I. W., B.Sc., Ph.D., Experimental and Research Station, Cheshunt, Herts.

1938 Shaw, H., M.Sc., Ph.D., East Malling Research Station, nr. Maidstone, Kent. (Secretary 1946– ; Assistant Secretary 1945; Council 1943–4.)
1937 Sheard, Mrs E., B.Sc., Experimental and Research Station, Cheshunt, Herts.
1932 Sheffield, Miss F. M. L., D.Sc., Ph.D., F.L.S., Rothamsted Experimental Station, Harpenden, Herts.
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1942 Sidwell, R. W., N.D.H., Knowle Hill, Badsey Lane, Evesham, Worcs.
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1946 Smith, I. C., B.Sc. Imperial Institute, South Kensington, London, S.W. 7.
1919 Smith, J. Henderson, M.B., Ch.B., B.A., Hill Harden, Harpenden, Herts. (President 1936–7; Treasurer 1931–9.)
1920 Smith, K. M., D.Sc., Ph.D., F.R.S., Director, Plant Virus Research Station, School of Agriculture, Cambridge.
1927 Smith, Prof. N. J. G., M.A., B.Sc., Ph.D., Rhodes University College, Grahamstown, South Africa.
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1919 Speyer, E. R., M.A., Experimental and Research Station, Cheshunt, Herts. (Council 1930–2, 1937–9.)
1920 Spinks, G. T., M.A., Research Station, Long Ashton, nr. Bristol.
1946 Stanton, W. R., B.Sc., Research Station, Long Ashton, nr. Bristol.
1944 Stapley, J. H., B.Sc., A.R.C.S., School of Agriculture, Cambridge.
1933 Steer, W., M.A., 3 Granville Road, Timperley, Cheshire. (Council 1942–4.)
1922 Stirrup, H. H., M.Sc., Midland Agricultural College, Sutton Bonington, Loughborough, Leics.
1919 Stone, H., Messrs Benton and Stone, Bracebridge Street, Birmingham. (Treasurer 1904–9.)
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1946 Street, H. E., B.Sc., Ph.D., Botany Department, University College, Nottingham.
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1914 Taylor, H. V., O.B.E., V.M.H., D.Sc., Ministry of Agriculture and Fisheries, Block 4, Bickenhall Mansions, London, W. 1. (Council 1927–8.)
1939 Taylor, R. E., B.Sc., Ph.D., School of Agriculture, Cambridge.
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1927 Thaysen, A. C., Ph.D., The Glaxo Laboratories, Greenford, Middlesex. (Council 1933–5.)
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1938 Thompson, H. W., M.Sc., Department of Agriculture, The University, Leeds 2.
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